Investigations towards the Synthesis of Isotope Labelled Analogues of Tocopherols and Tocotrienols

by

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List of Abbreviations

Ac	acetyl
aq	aqueous
Bn	benzyl
Bu	butyl
^{13}C-NMR	carbon nuclear magnetic resonance
dba	dibenzylideneacetone
DBU	1,8-diazabicyclo[5,4,0]undec-7-ene
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DMF	N,N-dimethylformamide
DMSO	dimethylsulfoxide
FAB/MS	fast atom bombardment mass spectrometry
IBX	2-Iodoxybenzoic Acid
IR	infrared spectrometry
NBS	N-bromosuccinimide
NIS	N-iodosuccinimide
PTC	phase transfer catalyst
RT	room temperature
TBAF	tetrabutylammonium fluoride
TBDMS	*t*-butyldimethylsilyl
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl

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Abstract

Vitamin E is considered as the most effective lipophilic chain breaking antioxidant. α-Tocopherol and its analogues have been studied thoroughly with regards to its biokinetics and bioavailabily. Deuterated tocopherols have been synthesized and utilized in such studies. Tocotrienols are arousing more and more interest because of their high efficiency as antioxidants. However, to date, there is no effective synthetic method reported for deuterated tocotrienols.

This thesis is focused on the investigation of the synthetic methods of deuterated tocotrienols and their analogues: 5-trideuteromethyl-α-tocotrienol, 5-trideuteromethyl-β-tocotrienol, tocotrienol acetate, silyl tocotrienol ether, etc. Several synthetic procedures for the preparation of poly-deuterated tocopherols are known. Mainly the deuterium is introduced by use of labelled formaldehyde and deuterated hydrogen chloride under Lewis acid catalysis. However, these methods are not effective in tocotrienols due to exchange of protons for deuterium at other sites under the acidic conditions. We developed several different approaches to generate polydeuterated tocotrienols by using both morpholinomethylation followed by reduction with $NaCNBD_3$ as deuterated reducing reagents and transmetalation strategy. The 5-trideuteromethyl-α-tocotrienol was finally obtained in a satisfactory yield of 60%. In addition, this thesis also discussed the study of structural comparison and the chemical property difference of tocopherols and tocotrienols, which provides hints to explain the reactivity difference of them towards oxidation at the C3-C4 positions.

Furthermore, the methodology of halogenation and dehydrohalogenation of tocotrienol was explored to prepare a hexaene tocotrienol derivative as a florescent reporter of tocopherol.

1. Introduction

1.1 Discovery and History of Vitamin E

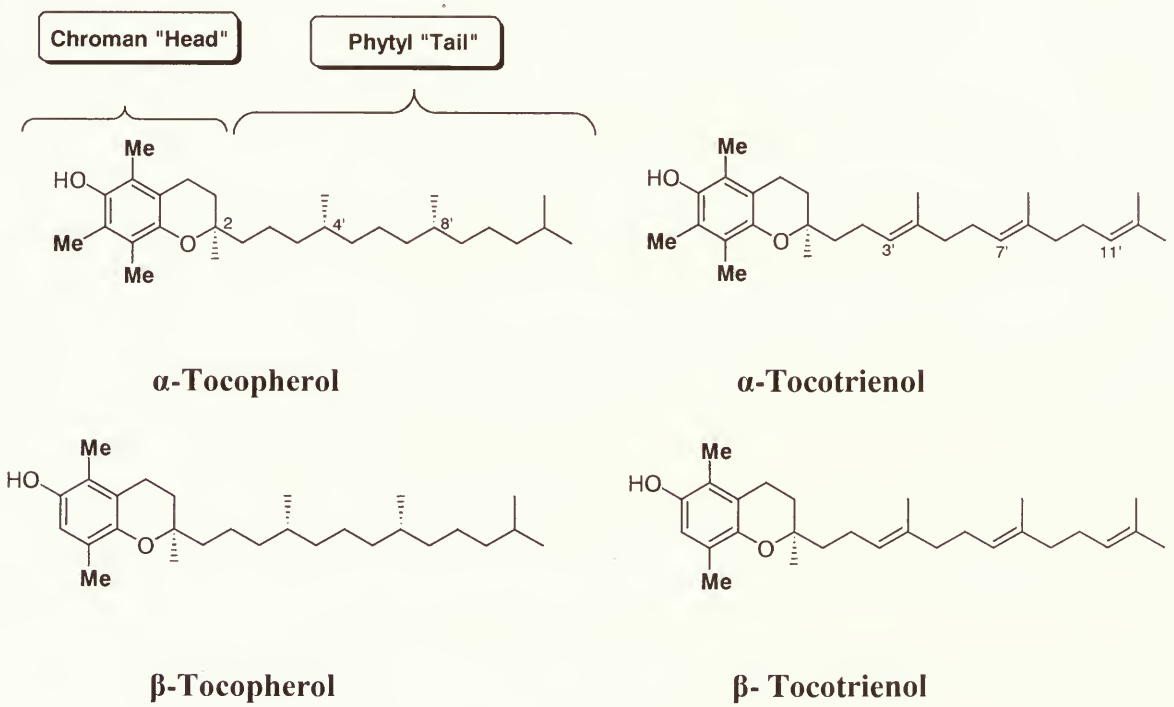
Vitamin E was first described as a dietary factor in animal nutrition in 1922 by Evans and Bishop [1] at the University of California, Berkeley, USA. A deficiency syndrome in female rats was observed after they were fed rancid fat. The most characteristic symptom of this syndrome was foetal resorption which was found to be reversed by adding fresh vegetables to the rat's diet. It was thus concluded that these observations resulted from a specific factor contained in plants which was later designated as Vitamin E by these pioneering researchers [2].

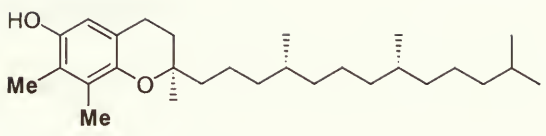
Evans isolated and characterized two compounds from wheat germ oil with vitamin E activity in 1936 [3]. These were named α– and β–tocopherol, from the Greek "*tokos*" (meaning childbirth) and "*phorein*" (meaning to bring forth). Later on, two more tocopherols, γ- and δ-tocopherol were isolated from plants oils [4, 5]. An additional four forms of vitamin E exist in the tocotrienol family, having the same variation in methyl group substitution on the chroman, but three degrees of unsaturation in the side chain at 3', 7', and 11' [6]. In 1989, the American Food and Nutrition Board of the National Research Council announced the Recommended Dietary Allowance (RDA) of vitamin E: 15mg of α–tocopherol equivalents to meet the requirements and prevent deficiency symptoms in normal humans [7].

1.2 Structures and Sources of Vitamin E

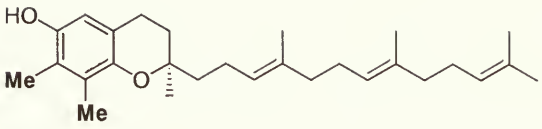
The four tocopherols and four tocotrienols (divided as two groups) are closely related in structure. All the compounds of both groups are 6-chromanol derivatives that have a chroman "head" and a phytyl "tail" (Figure 1). The first groups of compounds, the tocopherols, carry a saturated isoprenoid C-16 side chain bearing three chiral centers with the *R*-configuration at positions 2, 4′ and 8′. Tocotrienols, the members of the second group, have a triply unsaturated side chain at positions 3′, 7′ and 11′. Within each group, α, β, γ, δ are designated to the members according to the number and the position of the methyl groups attached to the aromatic ring.

Figure 1. Structures of naturally occurring components of vitamin E.

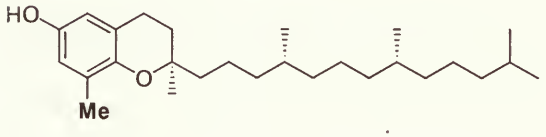




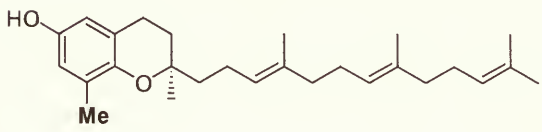
γ-Tocopherol



γ-Tocotrienol



δ-Tocopherol



δ-Tocotrienol

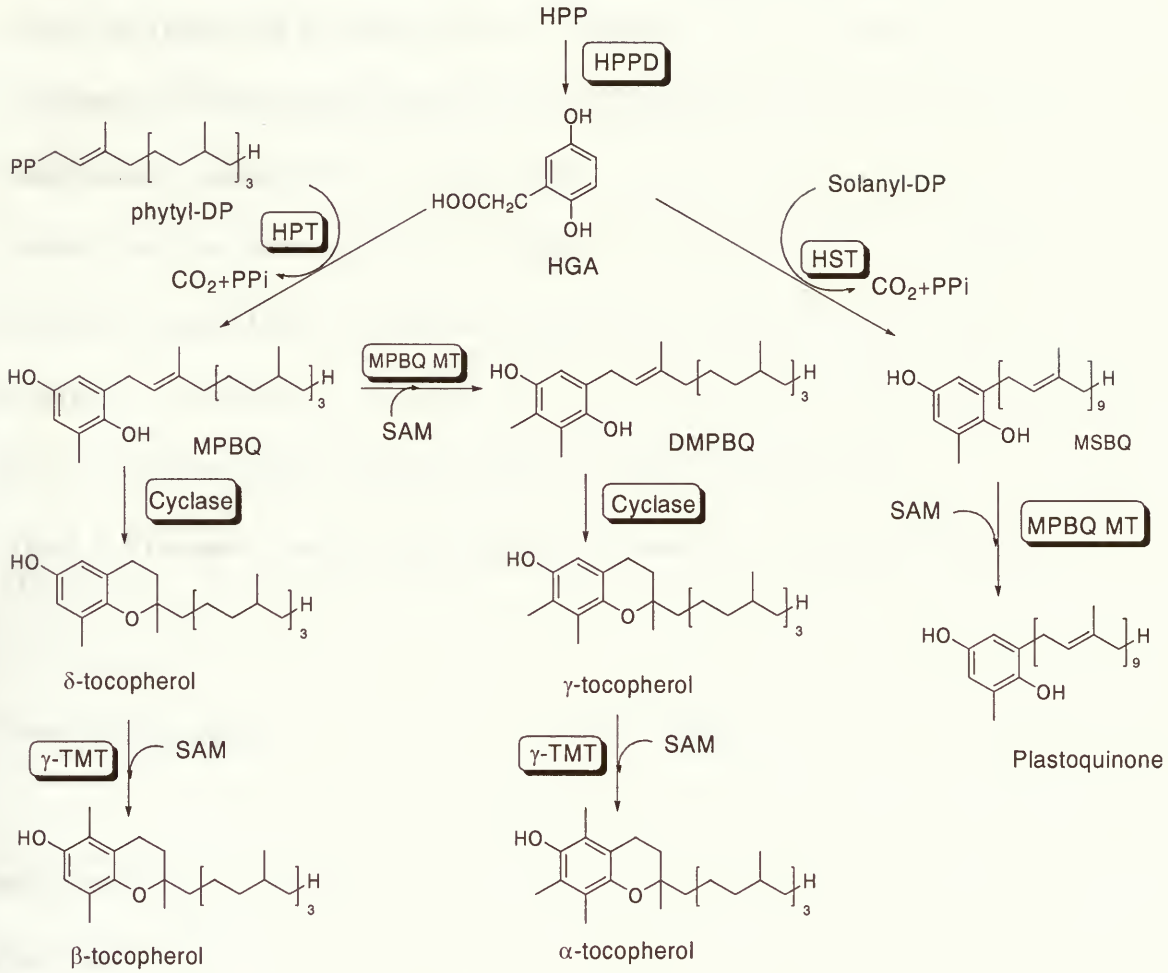
In nature, vitamin E is biosynthesized in plants, and especially rich sources are edible vegetable oils (Table 1) which contain all four tocopherols in varying proportions. Wheat germ oil, sunflower oil and safflower oil are rich in *RRR*-α-tocopherol; soybean and corn oils contain mainly γ-tocopherol as well as some tocotrienols; both palm oil and cottonseed oil contain equal proportions of α- and γ- tocopherol, while the former also contains large amount of α- and γ-tocotrienols. [8] Another good source of vitamin E is unprocessed nuts and cereal grains. Meat contains smaller amounts, as do fruits and vegetables.

Vitamin E is synthesized solely in plants. The biosynthetic scheme in Figure 2 shows the separate derivation of the aromatic ring and the isoprenoid side chain. [9, 10] The head group of vitamin E is synthesized through cytosolic aromatic amino metabolism; and the hydrophobic tail is synthesized through plastidic deoxyxylulose 5-phosphate pathway.

The important step in head group synthesis is the production of homogentisic acid (HGA) from *p*-hydroxyphenylpyruvic-acid (HPP) by *p*-hydroxyphenylpyruvic acid dioxygenase (HPPD). Prenylation of HGA yields the key intermediates in tocopherol and tocotrienol synthesis, 2-methyl-6-phytylplastoquinol (MPBQ) and 2-methyl-6-geranylgeranylplastoquinol (MGGBQ), respectively. MPBQ and MGGBQ are substrates for either tocopherol cyclase or MPBQ methyltransferase (MPBQ MT). MPBQ MT adds a second methyl group to MPBQ to form 2,3-dimethyl-5-phytyl-1,4-benzoquinone (DMPBQ) or to MGGBQ to form 2,3-dimethyl-5-geranylgeranyl-1,4-benzoquinone (DMG DMGGBQ).

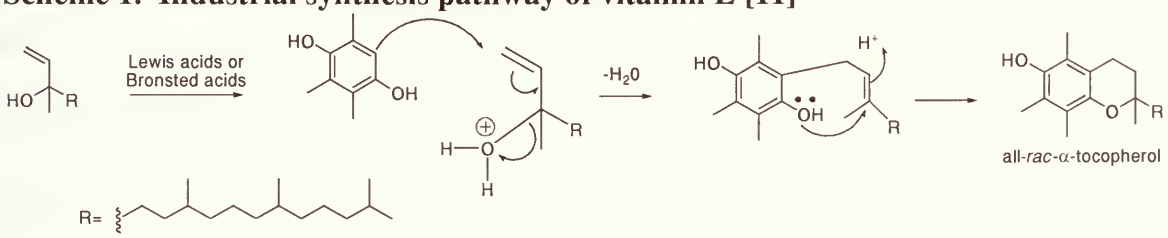
Tocopherol cyclase converts MPBQ and DMPBQ to δ- and γ-tocopherols, respectively, and corresponding geranylgeranylated intermediates to δ- and γ-tocotrienols. Finally, γ-tocopherol methyltransferase (γ-TMT) adds a methyl group to C-6 of the chromanol ring, converting δ- and γ-tocopherols and tocotrienols to β- and α-tocopherols and tocotrienols, respectively. [9, 10]

Figure 2. Biosynthesis pathway of vitamin E [9]



In industry, tocopherols are synthesized by the condensation between 2,3,5-trimethylhydroquinone and isophytol under Lewis acids catalyzed conditions, followed by hydrogenation and dehydration to form all-*rac*-α-tocopherols (Scheme 1). [11]

Scheme 1. Industrial synthesis pathway of vitamin E [11]



The synthetic form of vitamin E is used in most human vitamin supplements and animal nutrition and is called *all-rac*-α-tocopherol. It is synthesized by the condensation of trimethyl hydroquinone with isophytol [12] producing all eight stereoisomers arising from the three chiral centers. Due to their higher air stability over the corresponding free phenol, α-tocopheryl esters are marketed as the most common form of vitamin E supplements. After ingestion, the esters are hydrolyzed to the free phenols which are the active form accounting for vitamin E's antioxidant activity of which more details will be discussed below.

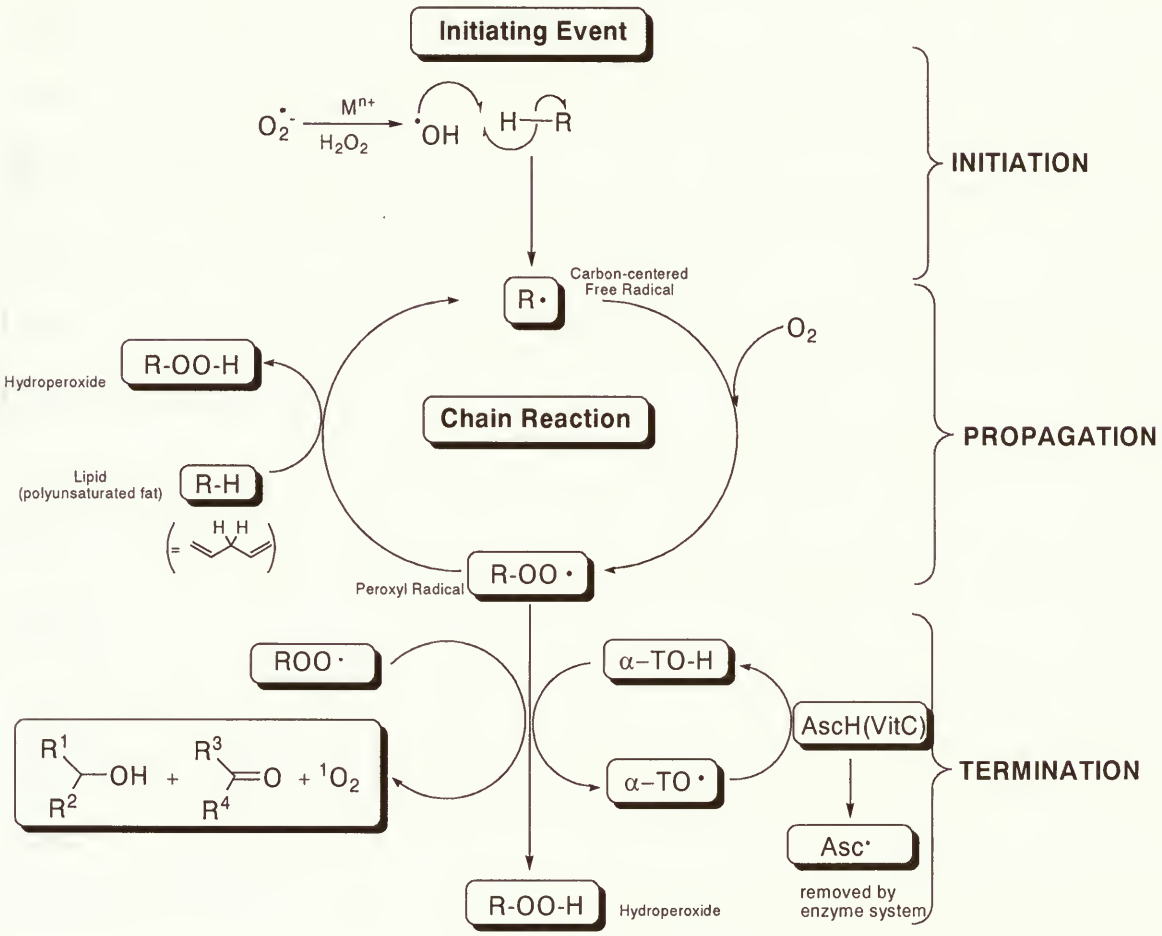
Table 1. Vitamin E content of selected foods (based on α-tocopherol content). [13]

Food (100g portion)	**Vitamin E**	
	mg	**IU (1 mg α-Toc =1.49IU)**
Wheat germ oil	119	178
Sunflower oil	49	73
Peanut oil	19	28
Safflower oil	40	59
Soybean oil	8.1	12
Margarine, hard	11	16
Margarine, soft	14	21
Butter	2.2	3.2
Sunflower seed, raw	50	74
Almonds	27	41

1.3 Antioxidant Activity of Vitamin E

Lipid peroxidation is the most common indicator of the operation of free radical processes in living systems [14]. Figure 3 shows the lipid peroxidation process as a chain reaction proceeding in three stages.

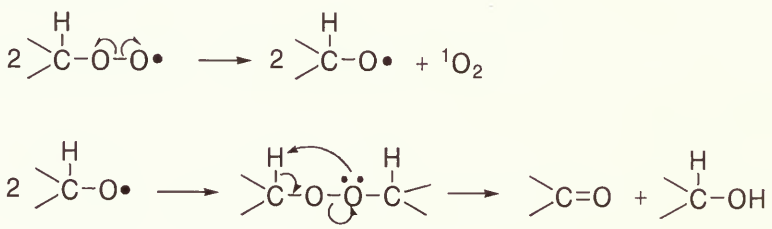
Figure 3. Free radical chain mechanism of lipid peroxidation. [14, 15]



In the initiation stage, carbon-centered lipid radicals, R•, are produced by the abstraction of a hydrogen atom by a reactive oxygen species of varying kinds, including peroxyradicals from previous lipid oxidation events. In the propagation

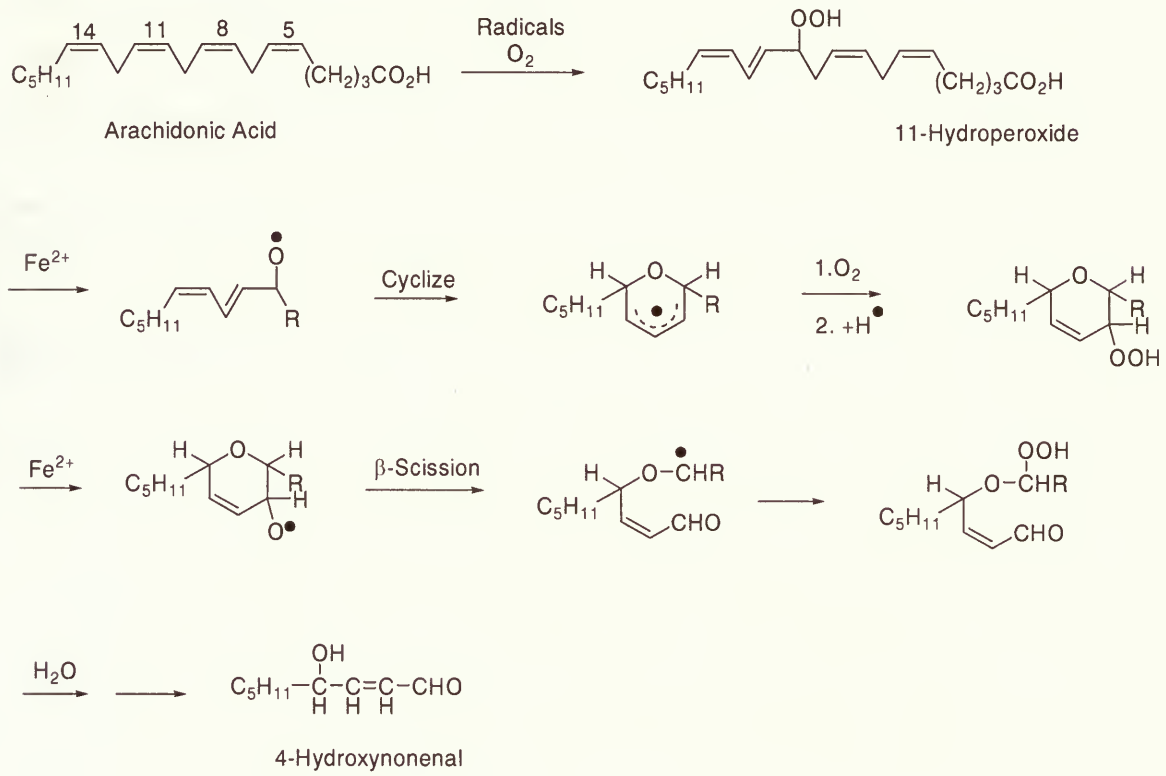
stage, the carbon-centered radical reacts rapidly with molecular oxygen to form a peroxyl radical ROO•, a chain-carrying radical that is able to attack another polyunsaturated lipid molecule. This peroxyl radical is converted to a hydroperoxide ROOH after it abstracts a proton from a second RH, which generates a new carbon-centered radical which can be involved in the same reactions as described above, illustrating the chain reaction nature of this chemistry. As this propagative process continues, valuable polyunsaturated fat is consumed and converted to a corresponding quantity of hydroperoxide. The chain reaction does not stop until the chain-carrying peroxyl radical (ROO•) abstracts an hydrogen atom from tocopherol to form an hydroperoxide; or two peroxyl radicals react with each other to generate inactive products as indicated in Figure 3 at the termination stage. Scheme 2 indicates the likely mechanism of the formation of ketone and alcohol along with singlet oxygen $^1O_2$ when two peroxyl radicals encounter each other. This is an effective chain termination reaction of lipid peroxidation, since it removes two peroxyl radicals. However, this self-reaction of peroxyl radicals is unlikely to be a favored reaction until they have accumulated to significant levels within the membrane, for example, until the peroxidation is already severe.

Scheme 2. Mechanism of two peroxyl radicals encounter reaction in lipid peroxidation process



As shown in Figure 3, once the lipid was peroxidized, the resulting hydroperoxide could be decomposed to hydroxyalkenals in the presence of transition metal ions. Scheme 3 shows a suggested mechanism for the formation of 4-hydroxy-2-nonenal (HNE) from the peroxidation of arachidonic acid [16].

Scheme 3 Mechanism of the formation of 4-hydroxy-2-nonenal (HNE) from the peroxidation of arachidonic acid [17]

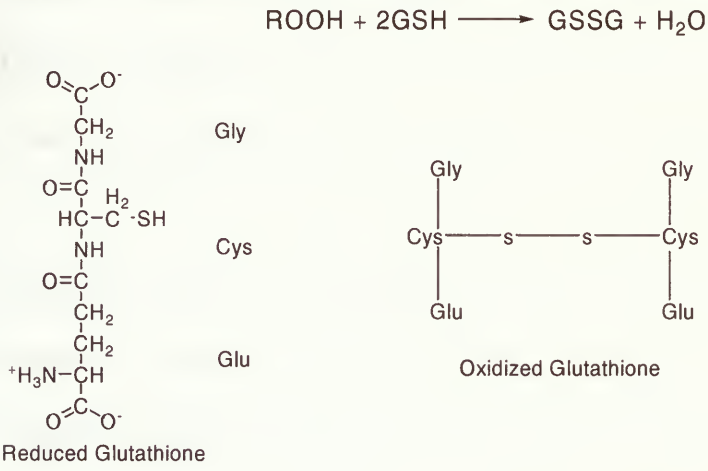


The generation of HNE within the membranes and lipoproteins can cause severe damage to the proteins present. The surface enzymes acting as receptors allowing cells to respond to hormones can be inactivated during lipid peroxidation. Potassium channels, essential for generation of electrical activity in nervous tissue and heart can be damaged, which can result in irregularities in

heartbeat and death of neurons [18]. The membrane fluidity is decreased by lipid peroxidation, making it easy for phospholipids to exchange between the two monolayers, increase the ease of transport of substances that do not normally cross it other than through specific channels and inactivate member-bound enzymes.[19]

When α-tocopherol quenches a peroxyl radical (ROO•), the resulting hydroperoxide ROOH can be converted to an alcohol by glutathione peroxidase. Reduced glutathione (GSH) can reduce a lipid hydroperoxide to an alcohol, while itself being oxidized to oxidized glutathione (GSSG) (Scheme 4) [20].

Scheme 4. Reduction of lipid hydroperoxide by glutathione (GSH)



It would be ideal if this undesired peroxidation process could be prevented and/or inhibited by stopping the initial formation of the radicals and/or by breaking the reaction chain at the propagation stage. Although, Vitamin E cannot prevent the formation of the radicals, it acts as an antioxidant to quench the chain carrying

peroxyl radicals ROO• so that the propagation process is interrupted, and by this means the radical reaction chain is broken. This is the reason why vitamin E is also called a chain-breaking antioxidant.

At the termination stage, vitamin E (α-tocopherol) reacts more rapidly with the peroxyl radical ROO• than polyunsaturated fatty acid (RH). The α-tocopherol (α-TOH) donates its phenolic hydrogen atom to the radical ROO• and converts it to a hydroperoxide ROOH. Inert enough to be unable to continue the reaction chain, the resulting tocopheroxyl radical is thought to be removed from the cycle by reacting with reducing agents, such as ascorbate (Vitamin C) or glutathione [14, 21]. The oxidized tocopherol (i.e. tocopheroxyl radical) needs to be recycled in order to be an effective antioxidant. It is currently believed that ascorbate (AscH) recycles tocopheroxyl radical producing the ascorbate radical (Asc•) which can be removed by dismutation and reduction by enzyme systems [21].

In living systems, it is difficult to obtain the direct demonstration of the actual extent of vitamin E in diminishing the lipid peroxidation. However, Lemoyne and coworkers found that the amount of pentane in the breath of humans supplemented with vitamin E is reduced [22]. This suggests that vitamin E prevents polyunsaturated fatty acid peroxidation since pentane is a minor product released during such reaction. Sharma reported the measurement of tocopheroxyl and ascorbate free radicals in plasma subjected to continuous oxidative stress using electron spin resonance (ESR) spectroscopy. An immediate increase in the

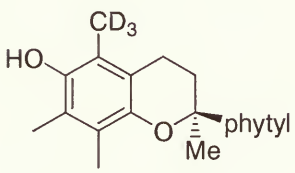
concentration of ascorbate radical was observed upon initiation of a continuous oxidative stress, which reached a peak, and then steadily declined. The appearance of the tocopheroxyl radical was observed only after disappearance of ascorbate radical. This data indicates that ascorbate is the auxiliary antioxidant of tocopherols [23]. It is also found that the chromanol head group of α-tocopherol is entirely responsible for the antioxidant properties; while the phytyl tail has no influence on the antioxidant activity. [24, 25, 26]

1.4 Biokinetics and Bioavailability of vitamin E

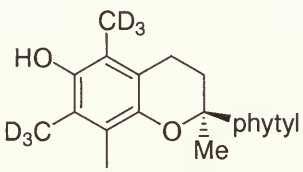
1.4.1 Deuterated α-tocopherol

Traditionally, either large doses of vitamin [27, 28, 29] or radiolabeled tocopherol [30, 31] were used in the *in vivo* studies of the absorption, transport and uptake of vitamin E. Both of these methods have their deficiencies. In the former case, because the large dose must be used to distinguish any change relative to background levels, the dose may not be physiologically relevant. In the latter case, the necessity of purification immediately before use due to the high tendency of radio- decomposition limits the application of the radiolabeled vitamin E. The use of stable isotope labeled tocopherols, combined with gas chromatography-mass spectrometry (GC-MS) has been applied to provide more accurate measurement of the absorption, transport, uptake and retention of tocopherol in humans and laboratory animals [14, 32]. By taking advantage of the availability of γ and δ-tocopherols and using the Lewis acid catalyzed methylation, investigators had prepared 2*R*, 4´*R*, 8´ *R*-α-tocopherol (*RRR*-α-tocopherol), the most abundant and naturally biologically active stereoisomer of vitamin E, containing three or six atoms of deuterium per molecule in the nonlabile, aromatic methyl positions or two deuterium atoms in the nonaromatic ring of the chroman head (of course this last compound was not made using Lewis acid catalyzed reactions.) (Figure 4) [32].

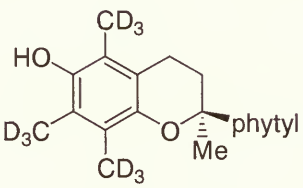
Figure 4. Structures of deuterated and silylated tocopherols referred to in the text. [14, 32, 33]



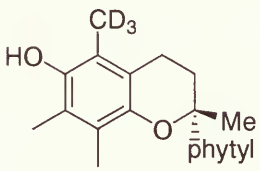
d_3-*RRR*-α-TOH



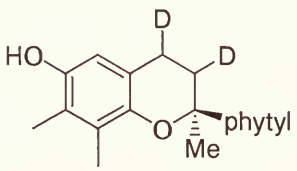
d_6-*RRR*-α-TOH



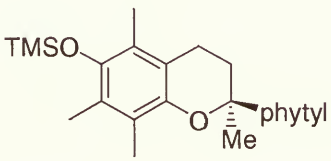
d_9-*all-rac*-α-TOH



d_3-*SRR*-α-TOH



d_2-*RRR*-γ-TOH



d_0-*RRR*-α-TOTMS

Deuterated tocopherols can be administered safely to animals and humans. Extraction and detection techniques have been developed to determine the relative amounts of deuterated (d_3- and d_6-α-TOH) and nondeuterated α-tocopherol (d_0-α-TOH) present in plasma, red cell and lipid extracts. The blood or tissue samples of rats or humans, dosed with deuterated tocopherols (d_3-*SRR*-α-tocopherol and d_6-*RRR*-α-tocopherol) as well as the nondeuterated tocopherols, are extracted with organic solvents containing a known amount of d_9-α-TOH, serving as an internal standard for the later GC-MS analysis. The tocopherol extracts are then derivatized as silyl ethers and injected into a GC-MS, where they are detected and identified by their characteristic parent m/z ions. By relating the peak area of these parent ions with that of the d_9-α-TOH internal standard, the absolute

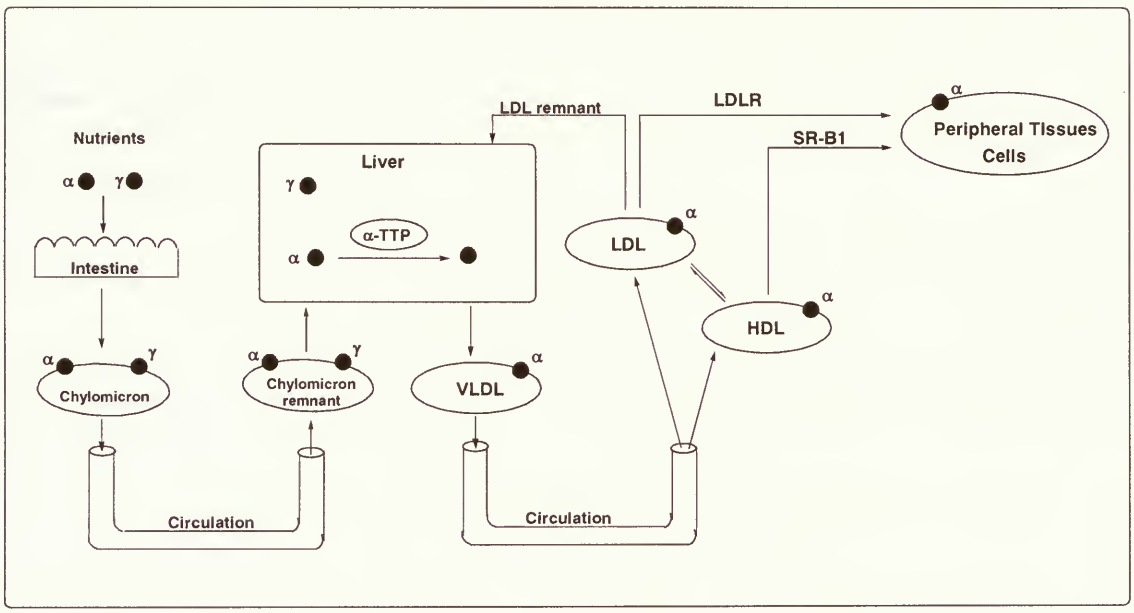
concentration of each tocopherol can be easily determined. The use of deuterated tocopherols has become a powerful tool for researchers conducting biokinetic and bioavailability studies of vitamin E [14]. For example, by giving subjects the oral dose containing equal amount of d_3-*SRR*-α-tocopherol and d_6-*RRR*-α-tocopherol, researchers found [34] that humans strongly discriminate these two forms of tocopherols with d_6-*RRR*-α-tocopherol preferentially secreted in very low density lipoproteins (VLDL). It was also found that this discrimination appears not to occur during absorption, but rather as a post-absorptive phenomenon in the liver. More details will be discussed in the next section.

1.4.2 Biokinetic Studies

As mentioned in Section 1.3, while the antioxidant activity of vitamin E is determined by the chroman "head", the biokinetics of vitamin E is largely determined by the stereochemistry at C-2 (R/S) and to some extent by the stereochemistry at C4′ and C7′. Because of this phytyl group, the resulting high hydrophobicity of vitamin E demands special mechanisms for the vitamin to be absorbed and transported in the aqueous environment of body fluids, plasma and cells. First taken up in the proximal part of the intestine, esterified vitamin E is hydrolyzed by pancreatic esterases to the free phenol which is then emulsified together with other fat-soluble components of food by bile salts and forms mixed micelles. These micelles are then absorbed at the mucosal membrane through passive diffusion and re-assembled, with phospholipids, cholesterol, apolipoproteins and triglycerides (fatty acid esters of glycerol), in the mucosa cell

to form lipoprotein aggregates called chylomicrons [35]. These chylomicrons are excreted by exocytosis to the lymphatic compartment from where they enter the blood stream within which the chylomicrons undergo intravascular degradation to chylomicron remnants by the endothelial lipoprotein lipase (LPL). This is now understood to be the non-specific mechanism for the absorption into the plasma and fast hepatic uptake of tocopherols [36]. (Figure 5)

Figure 5. Absorption, transport and distribution of vitamin E in human body [37].



Unlike the nonselective uptake of the vitamin E by the liver, a specific protein named α-tocopherol transfer protein (α-TTP) mediates the transfer of *RRR*-α-tocopherol, the biologically most active form of vitamin E, from the hepatic lysosomes into lipoproteins [37]. α-TTP exhibits a remarkable ligand specificity and selectively recognizes α-tocopherol out of all incoming tocopherols. Its ligand specificity and relative affinities of various tocopherol analogs have been

assessed and determined by Panagabko *et al* [38] and Hosomi *et al.* [39, 40]. Several structural features of α–tocopherol (Figure 1.) contribute to the specific ligand recognition by α-TTP: the three methyl groups on the chromanol ring, especially the methyl at position five (C-5) plays the most essential role for recognition; the hydroxyl group, the *R* configuration at C-2 as well as the orientation of the phytyl side chain are important determinants of tocopherol affinity to α-TTP. Panagabko and Hosomi reported the dissociation constants of α–TTP for different tocopherols and the relative α-TTP affinities of α-tocopherol and vitamin E analogs respectively (Table 2).

Table 2. Ligand selectivity of α-TTP.

Ligand	Dissociation constant (nM) of α-TTP [38]	Relative affinity of α - TTP for vitamin E [39,40]
α-tocopherol	25.0 ± 2.8	100
β-tocopherol	124 ± 4.7	38.1 ± 9.3
γ-tocopherol	266 ± 9	8.9 ± 0.6
δ-tocopherol	586 ± 75	1.6 ± 0.3
SRR-α-tocopherol	545 ± 62	10.5 ± 0.4

As a consequence of this selectivity, the homologues and non-natural isomers of α-tocopherol are excluded from the plasma and secreted with the bile [41]. The α-tocopherol is then incorporated into the very low density lipoprotein (VLDL)

fraction in the cytosol of the hepatocyte and transported into plasma to continue its journey in to remote tissues in the body.

Due to its hydrophobicity, α-tocopherol is transported in plasma only in association with lipoproteins. While all plasma lipoproteins can act as α-tocopherol carriers, it is the low- and high-density lipoproteins (LDL and HDL, respectively) that constitute the major vehicles of vitamin E in human plasma [42, 43, 44]. Through the mass transfer and redistribution of α-tocopherol among VLDL, LDL and HDL, α-tocopherol is delivered to different tissues and/or organs. LDL receptors (LDLR) and scavenger receptor class B type 1 (SR-B1) are believed to facilitate the uptake of α-tocopherol by peripheral tissues and cells from LDL and HDL respectively [34, 45].

1.4.3 Bioavailability Studies

Studies on the bioavailability of vitamin E in animals and humans are very important for a better understanding of its absorption and transport mechanisms. As mentioned in Section 1.2, vitamin E from natural and artificial sources such as synthetic all-racemic ones contains different stereoisomers, and one obvious question is "Do these two sources of vitamin function exactly the same in *vivo*?"

Many research efforts have been involved in the bioavailability studies of vitamin E to investigate the differences between the absorption and retention of two vitamin E stereoisomers (*RRR- vs. SRR*-α-tocopheryl acetate), between different

members of vitamin E (α-tocopherol *vs.* γ-tocopherol) and between different sources (natural vitamin E *vs. all-rac*-α-tocopherol). A method called the competitive bioavailability technique has been developed and adopted for such studies [14]. That is, each research subject is given a dose of a 1:1 mixture of two tocopherols to be compared and the relative uptake and transport of the two forms in the subject is monitored.

In the test of α-tocopherol *vs.* γ-tocopherol, it was found that although both were absorbed and secreted from the intestine in chylomicrons, α-tocopherol was preferentially incorporated into the VLDL secreted from the liver, resulting in the concentration of α-tocopherol in human plasma being higher than that of γ-tocopherol [14]. In the bioavailability studies of different vitamin stereoisomers, deuterated *RRR*- and *SRR*-α-tocopheryl acetates were used. It was found that chylomicrons contained similar amounts of d_3-*SRR*-α-TOH and d_6-*RRR*-α-TOH, while VLDL is preferentially enriched in d_6-*RRR*-α-TOH. This indicated that the discrimination in favor of d_6-*RRR*-α-TOH occurs during the secretion form the liver, but not during the absorption in the intestine. These observations are also consistent with the previously discussed function of the α-TTP which is mainly found in liver and is believed to be able to selectively recognize and bind α-tocopherol from other incoming tocopherols (Section 1.4.2, Figure 5). As for the bioavailability, research on natural vitamin E *vs. all-rac*-α-tocopherol concluded [46, 47, 48] that the natural vitamin E is between 1.36 to 2 times as potent. This suggests that the differences in bioavailability of natural and synthetic vitamin E

should be taken into account for individuals who choose to increase their vitamin E intake through supplementing their diets [49].

1.5 Relationship between Vitamin E and Disease

It has been suggested that oxidized LDL might have a role in the development of atherosclerosis by stimulating the formation of lipid-containing foam cells which are characteristic of early atherosclerotic foci in the vessel wall [8]. The oxidation of LDL, a process of lipid peroxidation, can be prevented by α-tocopherol. In vitro studies showed that, when enriched with tocopherol, the oxidation resistance ability of LDL increases proportionally with the tocopherol content [50, 51]. However, this is just a beginning for people to investigate vitamin E's potential function for atherosclerosis. Further studies are on going to explore vitamin E's implications for future application in such disease prevention. For more information on this topic, readers are pointed to references [52, 53, 54].

Investigations have shown that the level of antioxidant protection in the eye is a critical factor for the prevention of cataracts, one of the leading causes of blindness worldwide [55]. Researchers noticed that lower antioxidant status (vitamin E, vitamin C and carotenoids) is observed in subjects with senile cataracts than in those without [56]. It was suggested that in order to prevent the development of cataracts, resulting from oxidative stress in the epithelial cell layer, individuals should start vitamin E therapy well before the prevalence of maturity onset cataracts becomes significant. [57].

Once the vitamin E reaches the body tissues, it was found [14] that the neurological tissues (brain, cord, and nerve) are more reluctant to lose α-

tocopherol compared with other tissues (such as plasma, red cells, liver and spleen, etc.). This gives hints on the fact that vitamin E plays a very important role in maintaining neurological function in man. Long and severe vitamin E deficiency was believed [58] to cause the loss of nigrostriatal nerve terminals and oxidative stress may contribute the etiology of Parkinson's disease. The mechanism by which vitamin E performs this function remains to be determined, further studies might reveal its ability to minimize degenerative diseases. Ataxia with vitamin E deficiency (AVED) in human is also related to long term deficiency of vitamin E in diet. Common characteristics of the disease include progressive ataxia; clumsiness of the hands; loss of proprioception, especially of vibration and joint position sense. The treatment for AVED is lifelong high-dose oral vitamin E supplementation to bring plasma vitamin E concentrations to normal levels [59].

1.6 Tocotrienols

α-Tocopherol has been regarded as the most potent member in the vitamin family in terms of its bioactivity. However, more recent information indicates that tocotrienols exert more potency for the activities such as antioxidant, anti-cancer effects and cholesterol lowering, etc [60]. Very little work has been done with the biokinetics of tocotrienols. As shown in Figure 1, tocotrienols have three double bonds in the phytyl group, which offers the tocotrienols more fluidity and makes them much easier to be incorporated into the cell membrane [60]. Because of this property, tocotrienols are more effective in protecting the interior cell membranes, such as those that surround the cell nucleus and mitochondria. Accordingly, α-tocotrienol shows forty to sixty times more potency than α-tocopherol in the prevention of lipid peroxidation, while δ-tocotrienol is even more potent and is by far the most powerful antioxidant of the vitamin E family [60, 61].

In addition, tocotrienols are gaining more and more attention due to their anticancer effects. Tocotrienols can effectively promote an innate protecting process called apoptosis, an encoded suicide program preventing cells from becoming cancerous, with δ-tocotrienol being twice as potent as γ-tocotrienol [62]. Compared with the chemotherapy drug tamoxifen, γ-tocotrienol exhibits three times more potency in preventing the growth of human breast cancer by inhibiting the duplication stimulating enzymes within the cancer cell [63]. Furthermore, tocotrienols, especially δ-tocotrienol, show additional cardiovascular benefits by decreasing the amount of cholesterol plaque in arteries,

lowering the damaging lipoprotein level, preventing platelets aggregation, etc. [64].

Moreover, δ- and γ-tocotrienol also inhibit the liver enzyme HMG-CoA reductase, thus preventing the biosynthesis of cholesterol within liver [65]. It is found that the tocopherols have no cholesterol-lowering activity, but even worse, they can block δ- and γ-tocotrienol from inhibiting HMG-CoA reductase [66]. Over intake of tocotrienols can actually reduce their cholesterol-lowering ability. A suggested range of dosage of tocotrienol is between twenty five to one hundred milligrams per day [67].

1.7 Aims and Objectives

The synthetic work of this thesis will focus on the investigation of the first synthesis of multiply deuterated tocotrienols. Recently, Sen and his coworkers reported their study on the comparison of the efficacy of tocotrienols and tocopherols to protect against glutamate-induced death using neuronal cells. They found that tocotrienols were more effective than α–tocopherol in preventing glutamate-induced death. Uptake of tocotrienols from the culture medium was more efficient compared with that of α–tocopherol [68, 69]. Deuterated tocotrienols, with deuterium being the labeling agent, will be extremely useful in this type of research. Unfortunately, to date there is no published method about the synthesis of multiply deuterated tocotrienols although effective deuteration methods for tocopherols were reported almost two decades ago. In addition, amenable methods will be developed to afford d_x-tocotrienols in multi-gram scale for biological studies. Furthermore, preliminary research on the synthesis of novel unsaturated tocopherols with hexaene functionality in the phytyl side chain as a new fluorescent probe will be conducted and discussed in this thesis.

2. Results and Discussions

2.1 Synthesis of 5-Trideuteromethyl-α-tocotrienol

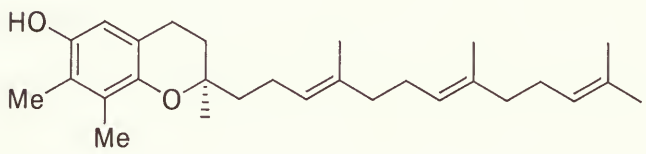
2.1.1. Brief Literature Review

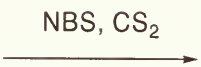
α-Tocopherol has been labeled with deuterium through attachment of a deuteromethyl group to the aromatic ring of a less-highly methylated tocopherol and elsewhere in the molecule during total synthesis [32, 70]. Deuterated α-tocopherols (Figures 3) have been successfully synthesized and utilized in the biokinetics and bioavailability studies of vitamin E [14]. The investigations on the bioavailability and metabolic fate of tocotrienols would be best carried out using a deuterium-labeled sample. However, the preparation of the deuterated tocotrienols encountered more serious challenges due to the unavailability of significant quantities of the required mono- and/or di-methyl tocotrienols from natural sources and the greater difficulties inherent in total synthesis of the *2R/S*-3'-*trans*-7'-*trans*-tocotrienol [71].

Until recently, the only publication on the subject of deuterium labeled tocotrienol was reported by Hyatt and his coworkers about the preparation of selectively deuterated γ-tocotrienol [72] (Scheme 5). γ-tocotrienol was treated with excess *N*-bromosuccinimide (NBS) in CS_2 at room temperature in the dark, and 5-bromo-α-tocotrienol was obtained in 55% yield after chromatography. The subsequent reaction of 5-bromo-α-tocotrienol with excess hydrazine-d_4 in D_2O/EtOD in the

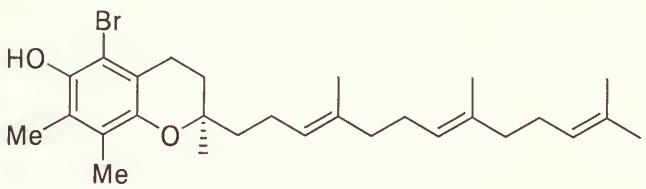
presence of 5% Pd/C catalyst gave the deuterium labeled 5-deutero-γ-tocotrienol in 60% yield.

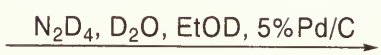
Scheme 5. Synthesis of 5-deutero-γ-tocotrienol



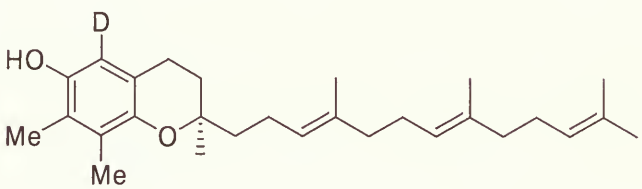


γ-tocotrienol





5-bromo-α-tocotrienol



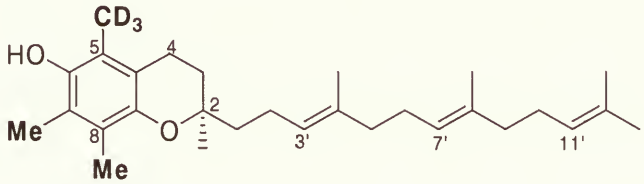
5-deutero-γ-tocotrienol

The major deficiency of this method is that the deuteration reaction does not go to completion, which leaves 25-30% unreacted 5-bromo-α-tocotrienol to be recovered. In addition, there is only one deuterium atom in the whole molecule, which makes it a rather poor probe for bioactivity studies because its mass

spectrum overlaps with the non-deuterated material. At present, there is no publication on the subject of deuterium labeled α-tocotrienol synthesis. The next few sections will focus on the endeavors in our laboratory on the first synthesis of 5-trideuteromethyl-α-tocotrienol.

2.1.2. Methodology developed in our laboratory

Instead of performing the multi-step total synthesis of tocotrienols [71], our laboratory initiated the investigation of deuterium labeling of natural-source γ-tocotrienol as a succinct route to our synthetic target, 5-trideuteromethyl-α-tocotrienol **1,** with three deuterium atoms in the molecule, which provides sufficient traceable analytical information in the bioactivity studies.



1

5-Trideuteromethyl-α-tocotrienol,

2R-3´-trans-7´-trans-γ-tocotrienol was obtained from commercial palm kernel oil product TOCOMIN® (purchased from Carotech Sdn Bhd.) which contains 50% (w/w) natural full spectrum tocotrienol/tocopherol complex including predominantly γ-, β-, δ-tocotrienol and α-tocopherol. Column chromatography was used to separate and purify these compounds for the later usage as starting

materials in the syntheses of deuterated tocotrienols. The purification result is shown in Table 3.

Table 3. Vitamin E ingredients separated from 2.02g TOCOMIN®.

Pure tocotrienols	α-tocotrienol	γ-tocotrienol	δ-tocotrienol
Amount obtained (g)	0.208	0.268	0.111
Percentage (%)	10.29	17.24	5.50

The mass spectra and the resonances in the ^{1}H-NMR spectra of these ingredients are identical to those of the synthetic tocotrienols published by Pearce [73]. Multi-gram scale tocotrienols isomers were separated from TOCOMIN® 50 oil. 30~35g TOCOMIN® 50 oil was purified on silica gel (4L) on a chromatography column (length = 100cm, diameter =10cm) using 10% ethyl acetate in hexane. The mass percentage of α-, δ-, γ- tocotrienol obtained from this large scale separation was α-tocotrienol 8-10% of the stating TOCOMIN® 50; γ- tocotrienol was 15-17%; δ-tocotrienol was 3-5% respectively. This percentage was similar to that obtained from the 2g- scale TOCOMIN® 50 oil separation.

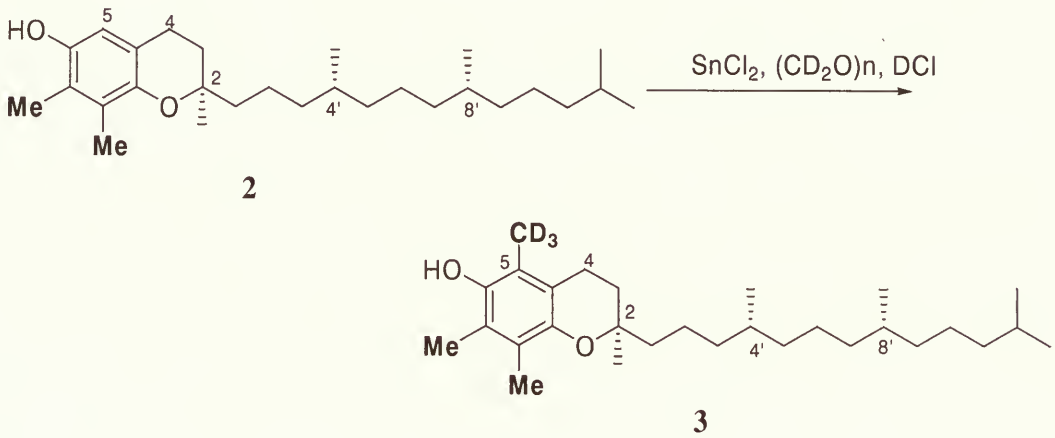
Method I: Lewis acid catalyzed d_3-methylation of γ-tocotrienol.

The preparation of 5-trideuteromethyl-α-tocotrienol initially adopted the Lewis acid ($SnCl_2$) catalyzed deuteromethylation of γ-tocotrienol with deuterated-paraformaldehyde [66, 67], which had been applied in the preparation of 5-CD_3-α-tocopherol and 5,7-$(CD_3)_2$-α-tocopherol from natural 2*R*,4′*R*,8′*R*-γ-tocopherol, and 2*R*,4′*R*,8′*R*-δ-tocopherol respectively [32, 70] (Scheme 6a, b). While no formal mechanistic studies have been done, a proposed mechanism is shown in

Scheme 6, c. The γ-tocotrienol prepared from the above TOCOMIN® product was used as the starting material (Scheme 6, d).

Scheme 6. Lewis acid catalyzed d_3-methylation of tocopherols and γ-tocotrienol

a.



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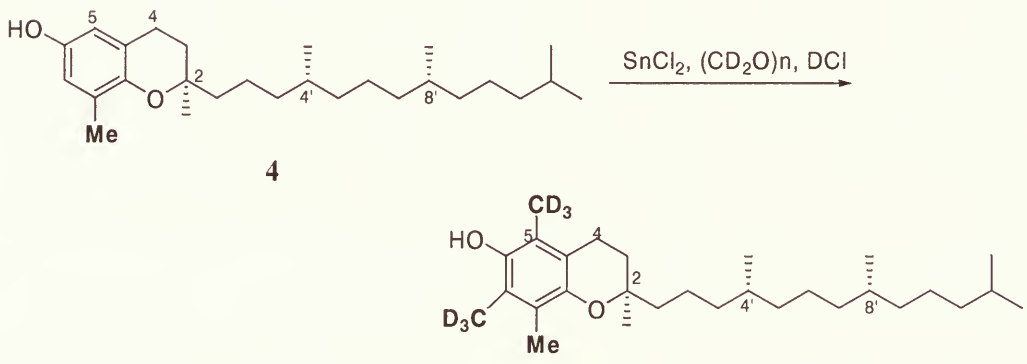
b.



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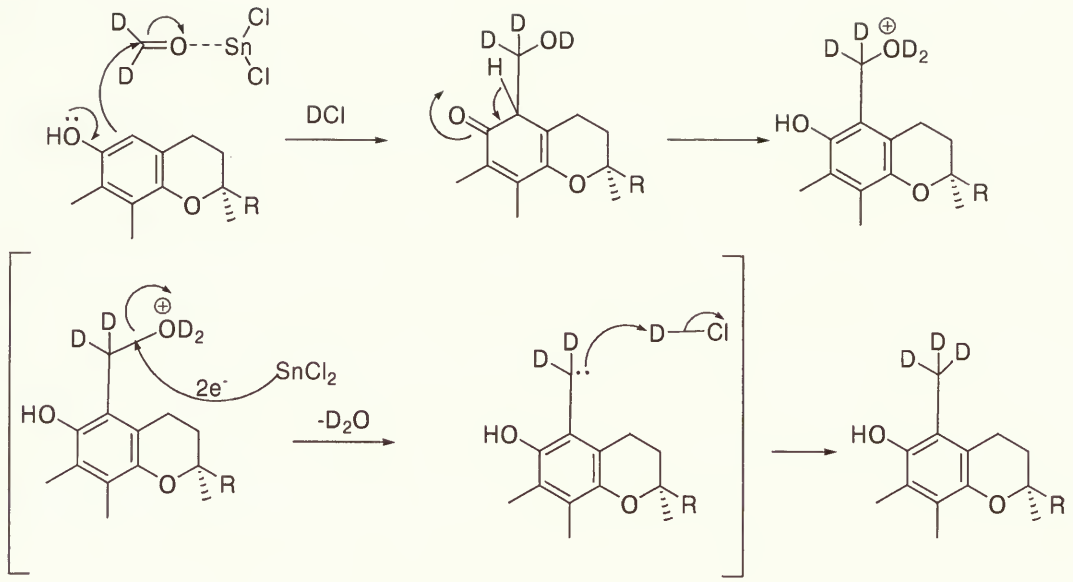
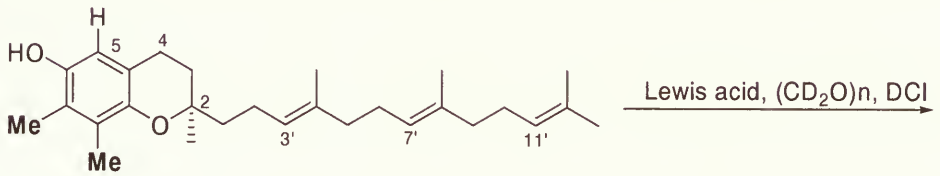
c.



d.



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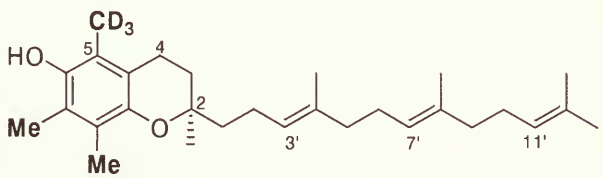


1

Many different conditions were attempted for this reaction using different equivalents of $(CD_2O)_n$, catalyzed by various amounts of $SnCl_2$ in the presence of excess DCl, as summarized in Table 4. With 2 to 4 equivalents of $SnCl_2$ at ambient temperature or heating at 40 and 60 degrees respectively in isopropyl ether, no reaction was observed after 7 to 48 hours (Entry 1-3). Increasing the amount of all the reagents, including $SnCl_2$, $(CD_2O)_n$ and DCl still afforded no desired product (Entry 4, 5). Due to the presence of the phytyl group,

tocotrienols have high hydrophobicity. Therefore, the phase transfer catalyst (PTC) Adogen464 (0.12eq) was used in some cases in order to enhance the mass transfer between organic and aqueous phases (Entry 1 and 5). Unfortunately no improvement in yield was observed. Upon refluxing in isopropyl ether for 5 hours, the desired product was obtained in a 27% yield (Entry 6). However, it was found that the reaction did not go to completion with some of the starting material, γ-tocotrienol remaining. This phenomenon was not observed in the case of tocopherols [74, 75].

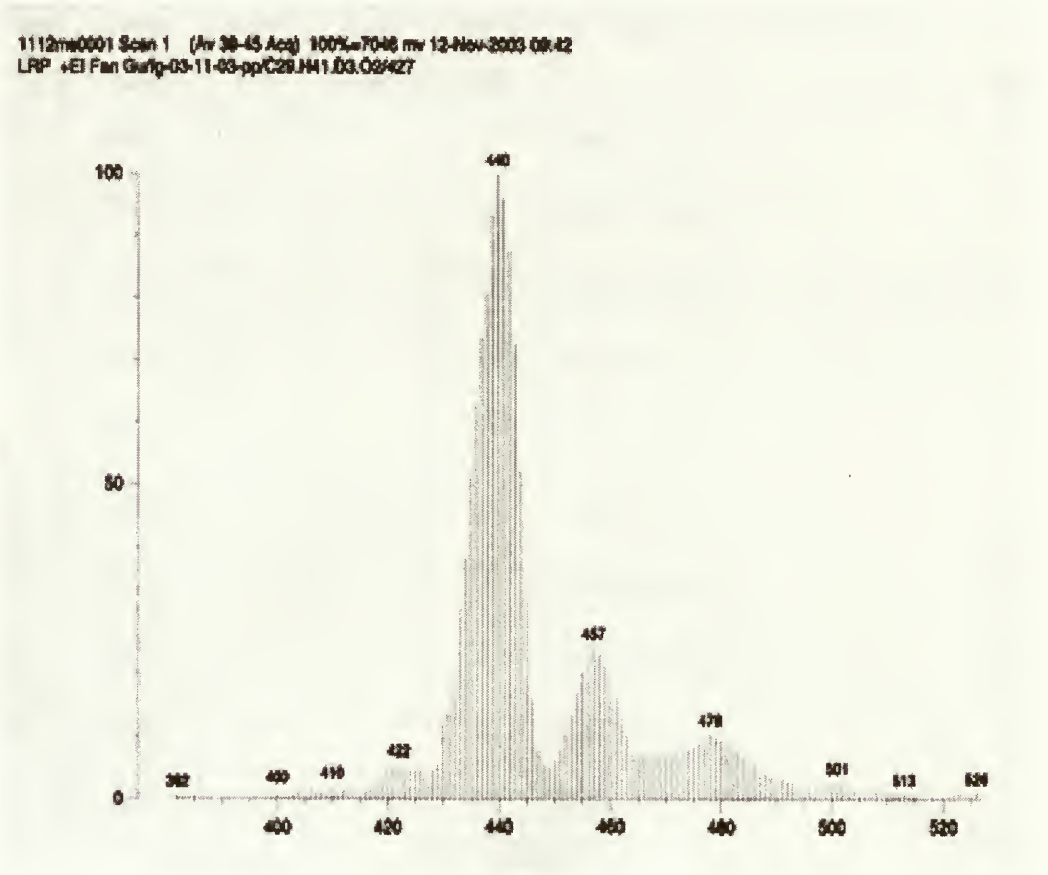
The evidence of product **1** formation was confirmed by the ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy. As shown in Scheme 2.d, it can be safely predicted that the C-5 aromatic proton signal in the ^{1}H-NMR spectrum of starting material γ-tocotrienol **6** should no longer exist in the spectrum of product **1** after the deuteromethylation. By comparing the ^{1}H-NMR (600MHz) spectra of **6** and **1**, the disappearance of the C-5 aromatic proton at δ=6.37 ppm on the spectrum of **1** can be clearly observed. Besides, it is also reasonable to foresee that in the spectrum of **1**, because of the existence of deuterium, the C-5 trideuteromethyl group would not show any signal as the regular methyl does in a proton NMR. This statement was supported through the comparison of the ^{1}H-NMR spectra of **1** and non-deuterated α-tocotrienol. In the spectrum of α-tocotrienol, there are three singlet signals at δ=2.17, 2.13 and 2.12 ppm respectively for the three methyl groups on the aromatic ring. In the product **1** spectrum, however, only two singlet signals at δ=2.16 and 2.12 ppm were observed. The lack of one singlet signal at

this region strongly supported the formation of product **1**. The intensities and chemical shifts of the rest of the signals in product **1** spectrum are identical to those of non-deuterated α-tocotrienol's. It is also apparent from the ^{13}C-NMR of product **1** that the deuterium aromatic methyl carbon appears at a lower field than that of the non-deuterated aromatic methyl carbons because of ^{13}C-^{2}H coupling. In the ^{13}C-NMR spectrum of non-deuterated α-tocotrienol, the three CH_3 carbons attached to the aromatic ring appear at 12.34, 11.91 and 11.40 as three consecutive singlet peaks with an equal interval (~ 0.5ppm); while in the ^{13}C-NMR spectrum of the product **1**, the CD_3 carbon at δ=10.93ppm, upfield than the other two aromatic CH_3 groups which are at δ=12.61 and 12.19 ppm respectively. The intensity of this CD_3 group is lower than of that of the nearby CH_3 group and its pattern appeared as a septet rather than the singlet of C-5 CH_3 group in the non-deuterated α-tocotrienol. This is consistent with the carbon-deuterium peak splitting pattern: number of peaks = 2NI+1 where N is the number of deuterium (N = 3 in our case) and I is the spin number of deuterium (I = 1). Therefore, the chemical shift and the peak pattern of the CD_3 group in product **1** positively confirmed the formation of 5-CD_3-tocotrienol. Furthermore, mass spectroscopy provided final confirmation for **1** by showing the ion at m/z of 427 for the molecular ion (M^+) of 5-trideutromethyl-α-tocotrienol, whereas the corresponding molecular ion (M^+) has a mass of 424 in the case of non-deuterated α-tocotrienol. This spectroscopic evidence proved that the 5-trideuteromethyl-α-tocotrienol **1** has been synthesized with a deuterium incorporation of d_3 92.1%, d_4 3.7%, d_5

4.2% based on the intensities of the M^+, M+1, and M+2 ions in the mass spectrum.

By using six equivalents of $SnCl_2$, four equivalents of $(CD_2O)_n$ and fifty-six equivalents of DCl, and prolonging the reaction time to 96 hours (Entry 7), it was found that instead of the normal deuterium incorporation of the deuteromethyl on the chroman moiety; much higher deuterium incorporation was observed from the mass spectrum (see Figure 6). The deuterium incorporation of the product is listed in Table 5 with the highest incorporation of d_{16} as 14.3%, while from its ^{1}H-NMR spectrum, no specific structure could be deduced. We assumed that under such reaction conditions, deuterium-hydrogen exchange might have happened to the substrate affording multiple deuterated products. Although the yield of this undesired product was 31%, better than previous reactions, it is not suitable for biological studies due to its multiple and non-specific placement of deuterium atoms.

Figure 6. MS spectrum of poly-deuterated α-tocotrienol



Other Lewis acids ($AlCl_3$ and BF_3) used for the methylation reaction with $(CH_2O)_n$ at various temperatures (-90°C to -10°C) also gave poor (5%) to moderate yields (34%) (Entry 8-13). No further reactions using $(CD_2O)_n$ were conducted based on the fact of unsatisfactory yields of the above attempts. $Yb(CF_3SO_3)_3$ [76] and $Sc(CF_3SO_3)_3$ [77] are known to be good catalytic Lewis acids for alkylation of aromatic compounds. Unfortunately, no reaction was detected when they were used in this case (Entry 14, 15). A similar result was experienced when the reaction was done with $ZnCl_2$ (Entry16).

Table 4: Summary of reaction conditions used in Method I.

Entry	Lewis Acid	Eq of L.A.	Eq of $(CD_2O)_n$	Eq of DCl	Solvent	t (°C)	Time (h)	Yield (%)
1[a]	$SnCl_2$	2	2	2	isopropyl ether	60	7	No rxn
2	$SnCl_2$	2	2	24	isopropyl ether	35-40	27	No rxn
3	$SnCl_2$	4	2	47	isopropyl ether	rt to 40	48	No rxn
4	$SnCl_2$	6	6	35	isopropyl ether	40	25	No rxn
5[a]	$SnCl_2$	6	4	35	isopropyl ether	50	3.5	No rxn
6	$SnCl_2$	4	1.56	27	isopropyl ether	Reflux	5	27
7	$SnCl_2$	6	4	56	isopropyl ether	Reflux	96	31[c]
8	$AlCl_3$	2	2[b]	---	dichloromethane	-78	5	21.5
9	$AlCl_3$	2	4[b]	---	dichloromethane	-18	3.3	29
10	$AlCl_3$	2	2[b]	---	dichloromethane	-10	3.5	5
11	BF_3	2	2[b]	---	dichloromethane	-15	3	34
12	BF_3	2	2[b]	---	dichloromethane	-78	5.5	24
13	BF_3	4	2[b]	---	dichloromethane	-90	6	26
14	$Yb(CF_3SO_3)_3$	2	2	25	isopropyl ether	r. t. to 70	12	No rxn
15	$Sc(CF_3SO_3)_3$	2	2.2	20	isopropyl ether	Reflux	12	No rxn
16	$ZnCl_2$	6	2	33	isopropyl ether	Reflux	117	No rxn

a. Phase transfer catalyst (PTC) Adogen (0.12eq) was used. b. $(CH_2O)_n$ was used instead of $(CD_2O)_n$. c. Yield of the perdeuterated product.

These results are quite surprisingly different from that of α-tocopherol d_3-methylation reported by Ingold *et al.*, of which the yield is generally at 60 to 70% [32]. The only difference between the two substrates is that there are three double

bonds existing in the phytyl group of α-tocotrienol, but not in the tocopherols. Obviously, the Lewis acid catalyzed d_3-methylation of α-tocotrienol did give us the desired product, but not with an acceptable yield. More effective methods still require further explorations.

Method II: Transmetalation strategy.

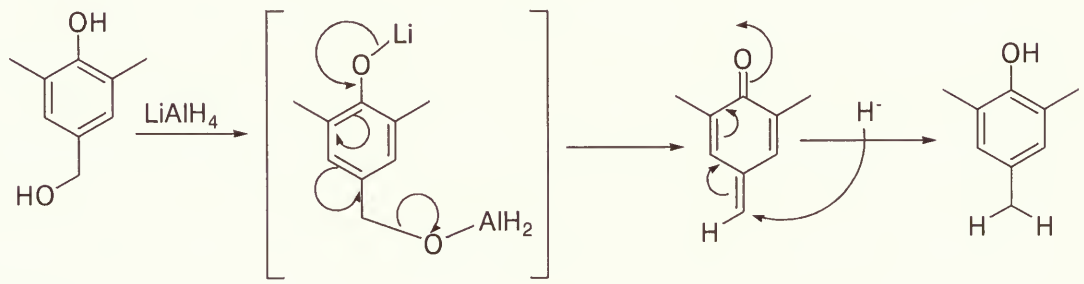
The above unsatisfactory yield of the d_3-methylation forced us to seek other methods to more efficiently introduce the d_3-methyl group to the *ortho* position of phenol in the chroman head of tocotrienol.

Several procedures have been reported for the C-methylation of phenols, among which the most common ones are the reduction of benzylic alcohols, aldehydes, and benzonitriles to produce the desired methyl groups. Baik and co-workers reported the lithium aluminium hydride promoted reductive deoxygenation of hydroxybenzyl alcohols to give the corresponding alkylphenols [78]. Benzoquinone methide was formed as a key intermediate (Scheme 7. a). The dehydroxylations of various *p*-hydroxybenzyl alcohols under similar conditions were studied and provided the corresponding *p*-methylphenols in reasonable yields. However, in the case of *o*-hydroxybenzyl alcohols, even though the formation of dehydroxylated products was the major reaction pathway, the dimerization of *o*-benzoquinone methides occurred to a significant degree.

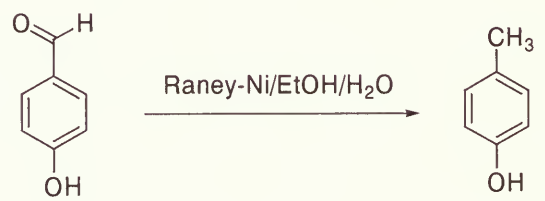
Aryl carbonyl compounds can be reduced under neutral conditions using Raney-Nickel in aqueous ethanol [79] (Scheme 7.b). Although such conditions did not affect the hydroxyl group, it seems not that convenient in our case where extra steps are needed to introduce the formyl group to the desired C-5 position in tocotrienol molecule. The existence of three double bonds at the tocotrienol side chain is also a concern for reductive conditions. The same sorts of considerations are taken into account if one were to use transformation of aromatic nitriles into the corresponding methyl derivatives with ammonium formate, reported by Ram and co-workers [80] (Scheme 7. c).

Scheme 7.

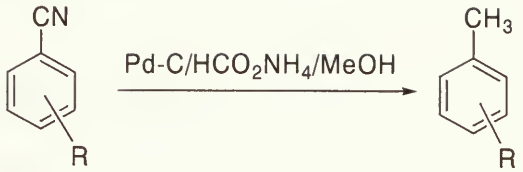
a.[78]



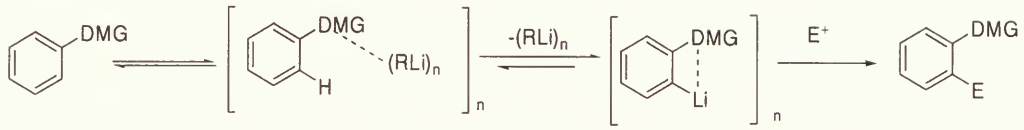
b.[79]



c.[80]



d.[81]



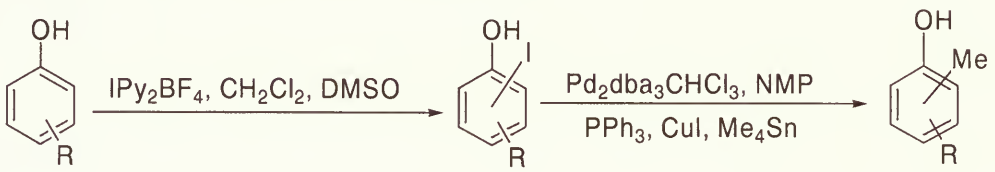
W X Y Z

Directed *ortho* metalation has also been applied for the C-methylation of phenols. Aryl C-methylation through directed ortho metallation (DoM) process is a three-step sequence (Scheme 7. d) [81]: the coordination of alkyllithium base $(RLi)_n$ aggregate to the heteroatom-containing directed metallation group (DMG), W ⟶ X; deprotonation to give the coordinated ortho-lithiated species, X ⟶ Y ; and reaction with an electrophile to yield product, Y ⟶ Z. The most common DMG's are OMe, $OCONR_2$ and $CONR_2$. The prerequisites for us to use this method are the transformation of the free phenol group to corresponding DMG and the deprotection to release the hydroxyl group after the ortho methylation. Therefore, this method would not hold the highest priority if we can find an alternative way to fulfil direct methylation without the need to protect the phenol hydroxyl group.

Stille [82] or Negishi [83] coupling reactions were also reported to perform the methylation of protected phenolic substrates. Recently Hudgens and his

coworkers published their result [84] of using the co-catalytic, palladium-copper Stille reaction [85] to convert various phenols to their corresponding C-methylated derivatives (Scheme 8). Phenols were converted to the desired methylated analogs by a two-step procedure. The phenols were first iodinated using Barluenga's reagent (IPy_2BF_4) to afford iodophenols [86] which were then converted to the methylated derivatives via a co-catalytic, palladium-copper Stille reaction.

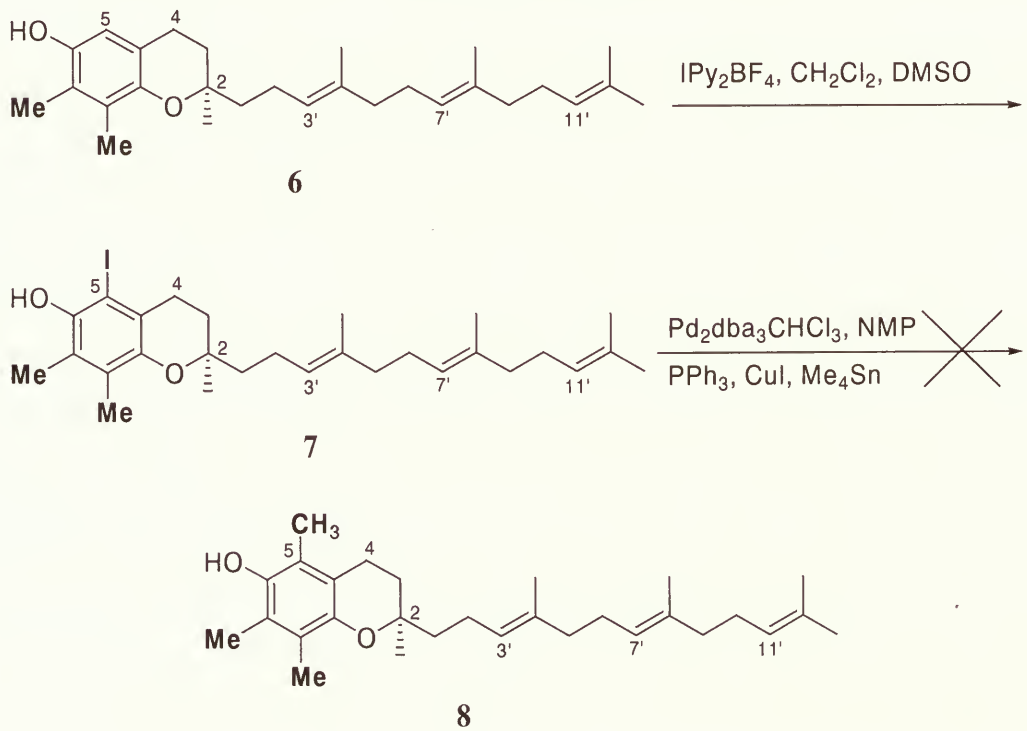
Scheme 8. Overview of phenol methylation



This method seemed promising for our case since: first, Barluenga reagent was known as an effective *ortho* iodination reagent for free phenols with generally excellent yields of over 80% [87]. The iodination of γ-tocotrienol appeared to be straightforward because there is only one aromatic proton (C5-H) *ortho* to the hydroxyl group. Second, this method has proven effective in the presence of other redox active functionalities and does not require protection of the phenol [84]. A new synthetic route was designed as shown in Scheme 9. Treating γ-tocotrienol with Barluenga reagent was followed by the palladium-copper Stille reaction using Me_4Sn as a model methylation reagent before the actual trideuteromethylation.

Upon the treatment of γ-tocotrienol with Barluenga reagent at ambient temperature for twenty minutes, the disappearance of the starting material was accompanied by the appearance of a non-polar spot with high R_f (R_f= 0.85, hexane/EtOAc 9:1) on thin layer chromatography (TLC). ^{1}H-NMR of the crude product showed the absence of the only aromatic proton in the molecule, indicating the loss of the proton at C-5. However, our attempts to isolate this product with column chromatography on silica gel resulted in its decomposition even when the column chromatography was conducted in dark. Preparative TLC was also used in order to isolate the product, but no desired iodination product was detected by the mass spectrum (MS).

Scheme 9. Iodination followed by methylation of phenol

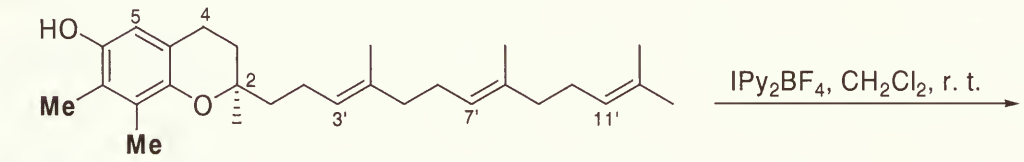


Due to the poor stability of the iodo intermediate, the crude mixture of the first step reaction was used directly in the subsequent methylation step without further purification. The crude intermediate was added to a high-pressure reaction tube containing N-methylpyrrolidinone. $Pd_2dba_3CHCl_3$ and triphenylphosphine were added, and the mixture was heated to 50°C for 10 min. Copper iodide was then added and the heating was continued for another 10 min. After cooling to room temperature, tetramethyl tin was added to the stirring solution. The tube was sealed at this stage and heated to 65°C overnight with stirring. Unfortunately, no desired product was observed by TLC after workup; only γ-tocotrienol remained. No further trideuteromethylation reactions were conducted using this method based on the unpromising result of the above model methylation reaction.

Another attempt was conducted with the expectation that the phenyllithium prepared from iodine-lithium exchange between iodo-γ-tocotrienol **7** and *t*-butyl lithium would react with methylation reagent to give the target product **1** (Scheme 10). Barluenga's regent was used to iodinate γ-tocotrienol **6** again to afford crude compound **7,** which was treated with TBDMS-triflate in 2,6-lutidine at 0°C to protect the hydroxyl group. The iodine-lithium exchange of the resulting 5-iodo-γ-tocotrienol silyl ether **9** with *t*-BuLi followed by trapping with deuteromethyl iodide produced **10**, which could be deprotected by treatment with tetrabutyl ammonium fluoride (TBAF) in tetrahydrofuran (THF) at 0°C. The final product **1** was obtained in an overall 10% yield from starting material γ-tocotrienol **6** in four steps. The ^{1}H-NMR and mass spectrum of the product obtained through this

transmetalation method (Method II) are identical to those from the previous Lewis acid catalyzed method (Method I). The unsatisfactory low yield of this method may result from the use of crude intermediates which we were unable to purify due to their poor stability and/or the failure to completely transmetalate compound **7**.

Scheme 10. Synthesis of compound 1 by using transmetalation approach



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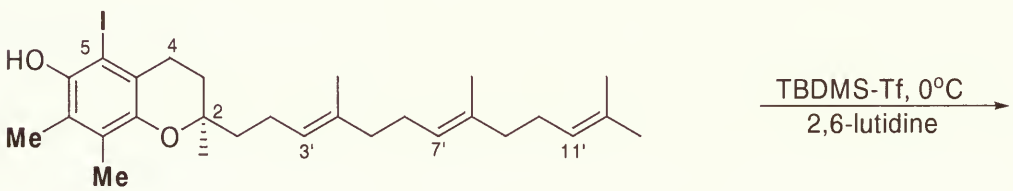

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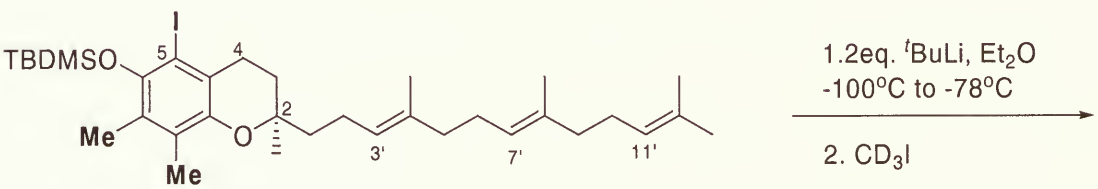

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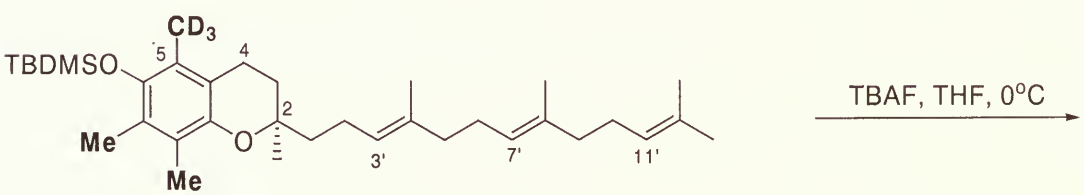

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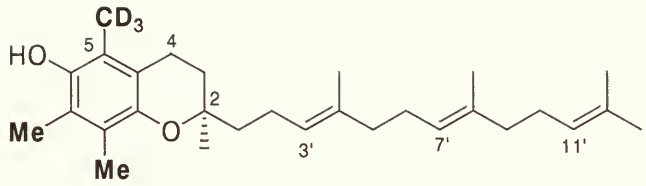


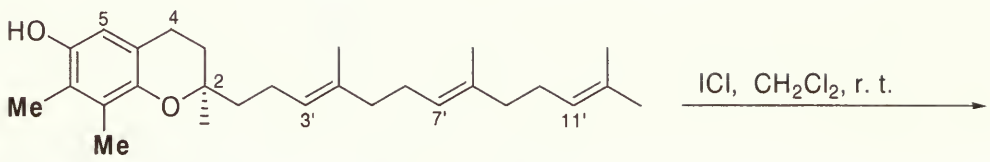
1

Other iodination reagents besides Barluenga reagent were utilized to introduce iodine to C-5 of γ-tocotrienol. The reaction of **6** with NIS in THF was run for various lengths of time. No desired iodination product was observed. When iodochloride was used, a dimerization product **11** connecting at C-5 position was found (Scheme 11a). In the ^{1}H-NMR of compound **11,** the aromatic C-5 proton signal was not observed while the rest signals are identical to those of γ-tocotrienol. In the mass spectrum of compound **11**, the molecular ion of dimer (M^+) = 818 was observed.

We also considered introducing the bromo instead of iodo group into the C-5 position, then converting the bromo compound into deuteromethyl product via methylation using d_3-iodomethane (Scheme 11b). The attempts with NBS in both THF and benzene did not afford the bromination product. While the reaction between γ-tocotrienol and pyridinium tribromide in benzene did give the desired product **13** with a C-5 bromo group, the subsequent d_3-methylation after phenol protection was unable to produce the 5-d_3-α-tocotrienol.

Scheme 11.

a.



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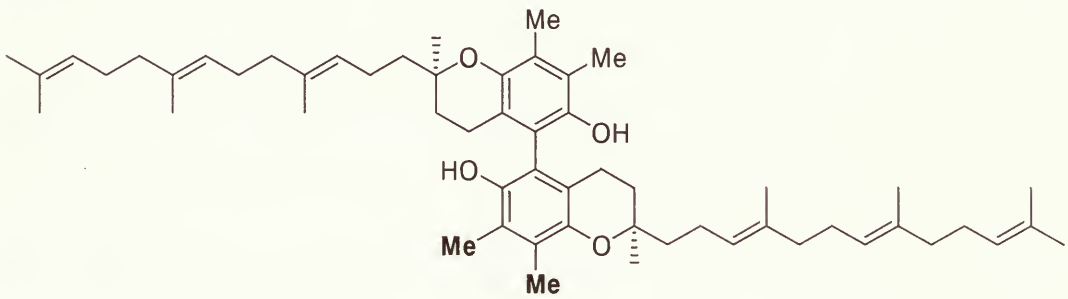

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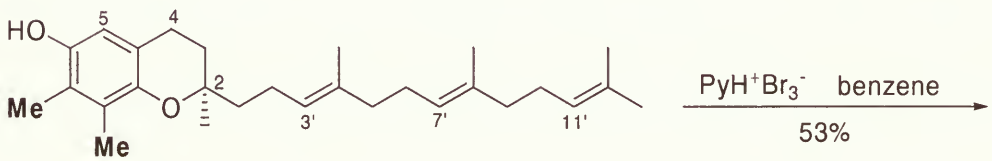
b.



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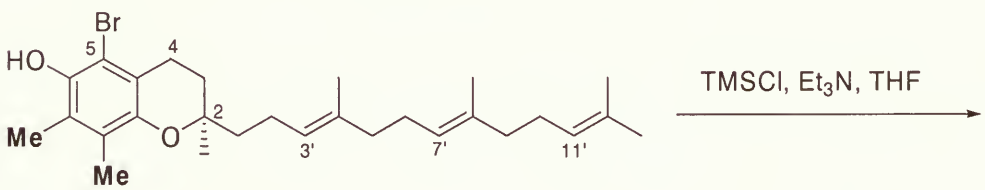

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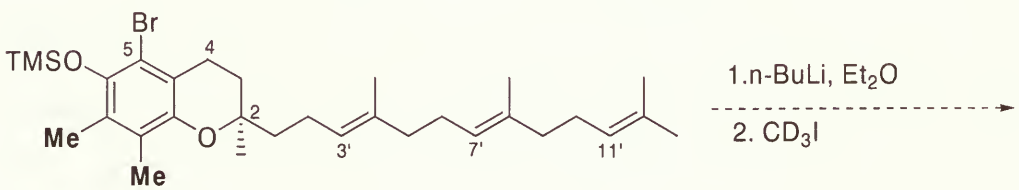

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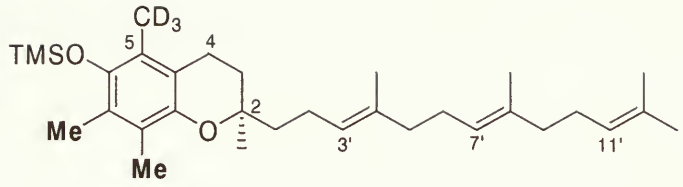

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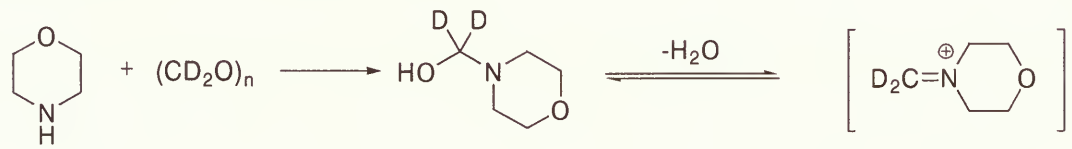
Changing the protecting group to methoxymethyl (MOM) group could be a possible alternative if this reaction would be attempted in the future by other researchers in our academic group. Clearly, a more efficient method is needed to make **1** in sufficient quantities for biological trials.

Method III: Synthesis via aminomethylation

In 1939, Caldwell *et. al.* reported the C-methylation of phenols and naphthols through aminomethylation using a Mannich base, and subsequent fission of the substituted benzylamine by catalytic hydrogenation [88]. The method also has been used for aminomethylation of tocopherols by Mueller and coworkers [89].

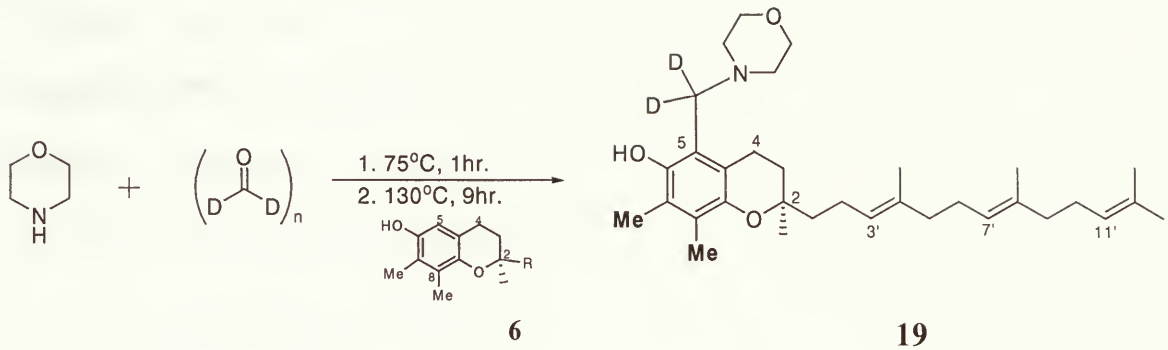
Scheme 12.

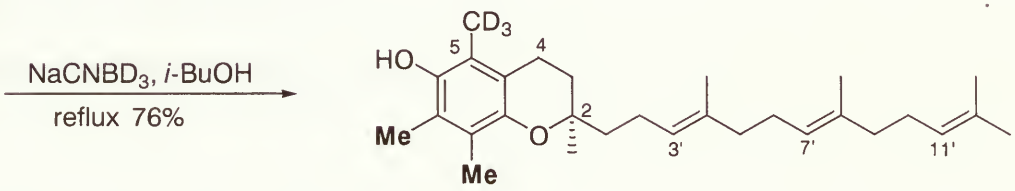
a. Preparation of Mannich base.



15 **16** **17** **18**

b. Synthesis of D_3- α-tocotrienol





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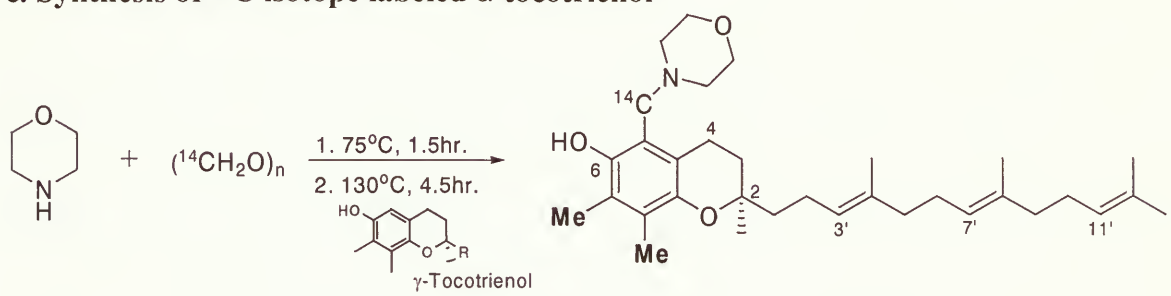
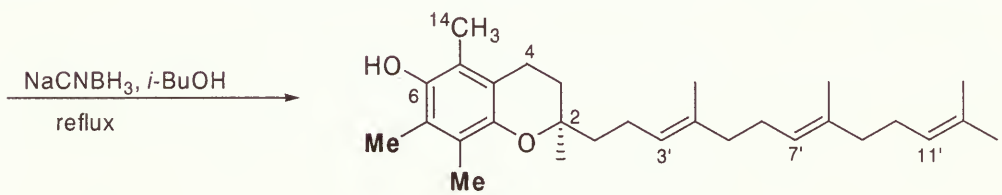
c. Synthesis of ^{14}C isotope labeled α-tocotrienol





Scheme 12 shows our version using deuterium labeled reagents. Mannich reagent was prepared *in situ* from perdeutero-*para*-formaldehyde and the secondary

amine morpholine in the absence of an additional solvent (Scheme 12 a). Neat γ-tocotrienol was then added to the reaction mixture, the aminoalkylated intermediate **19** was isolated in an 80% yield after heating at 130°C for 9 hours (Scheme 12b). In the ^{1}H-NMR of compound **19**, no aromatic proton was observed at δ=6.37 ppm, the chemical shift where the C-5 proton was found in the case of the unsubstrate γ-tocotrienol. In the mean time, the protons in the morpholine moiety can be found as two broad signals for two protons each at δ=3.79 ppm and δ=2.61 ppm. The EI MS showed a molecular ion of m/z = 511, which confirmed the formation of this key intermediate **19**.

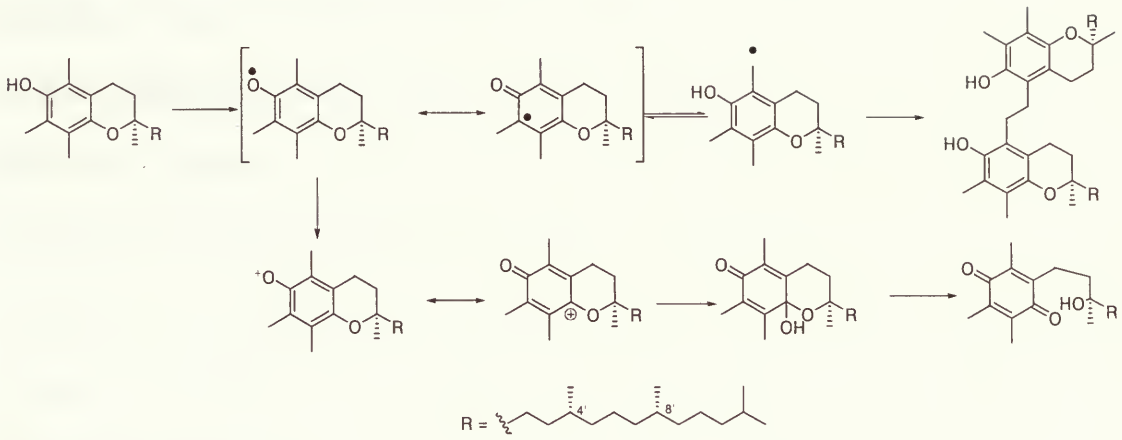
At this point, our work discussion with Dr. Thomas Netscher at Roche provided us with choice of solvent and temperature for best results. The deamination of **19** by conventional reduction with sodium cyanoborodeuteride in refluxing isobutanol affords product **1** in a 76% yield. Thus, the desired d_3-methyl-α-tocotrienol was successfully synthesized in a two-step method using Mannich reagent with an overall yield of 60%. This is, so far, the most efficient synthesis of this compound in an acceptable yield, and this convenient method provides a gram scale synthetic route for deuterated α-tocotrienol, which would bring good availability of this compound as a tracing agent in the biokinetic and bioavailability studies of tocotrienol.

This methodology (the above described amino methylation) also makes it possible to prepare radiolabeled α–tocotrienol by using ^{14}C-labeled *para*-formaldehyde

(Scheme 12, c). Milligram-scale (<3mg) reactions using non-radiolabled *para*-formaldehyde were completed successfully before the actual use of the ^{14}C isotopic reagent. The chemical yield was ~ 60% for the two steps. The first attempt using isotopic reagent was conducted by using 250 μCi (90 mCi/g, 2.78mg) of ^{14}C *para*-formaldehyde. Unfortunately, no desired product was observed other than some highly polar by-product according to TLC. It is possible that this occurred because of autoradiolysis from the high concentration of radiolabel in the reaction. We have experienced this previously during storage of stoichiometrically tritiated α–tocopherol (~59 Ci/mmol). If decomposition of a ^{14}C nucleus produces a radical, then this will produce a phenoxy radical from the starting material. High concentrations of phenoxy and other radicals will lead to coupling and ring opening.

With a half live of 5,700 years, ^{14}C decays into ^{14}N by releasing a β particle with the energy of 0.156 MeV. This energy released from the ^{14}C decay might have the ability to strip an electron from the tocopherol molecule to give a radical cation, which in turn could cause the formation of quinone or dimer (Scheme 13) in a similar oxidation pattern of tocopherol reported previous by Suarna and coworkers [90]. This could be a reason why with the high concentration of ^{14}C *para*-formaldehyde, the CD_3 methylation did not give the desired product.

Scheme 13. Radical decomposition of Vitamin E



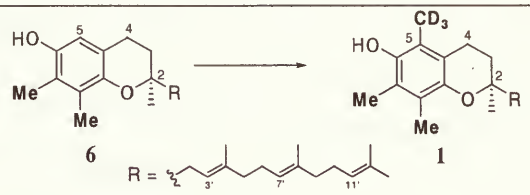
Thus, two separate model reactions using regular *para*-formaldehyde were done with large excess of morpholine and five equivalent of γ-tocotrienol respectively. The former reaction gave no desired product, while the latter give nice and clean product with acceptable yield (68%). Encouraged by this result, we started a second ^{14}C isotopic reaction using the same amount of radioactive reagent 250 μCi (1.9 Ci/g, 0.13mg) diluted in 2.6mg of regular *para*-formaldehyde. As stated above, five equivalents of γ-tocotrienol was used in the subsequent step, and 12.4 mg pure product **21** was obtained in 27% chemical yield with 82% radiochemical yield tested by scintillation counter. Product **21** was dissolved in 25 mL solvent mixture of 1:1 Toluene : EtOH. 2μL of this solution was taken for the radioactivity test by using scintillation counter and given the radioactivity value of 36578 DPM. Thus, the radioactivity value of the total product is calculated as following:

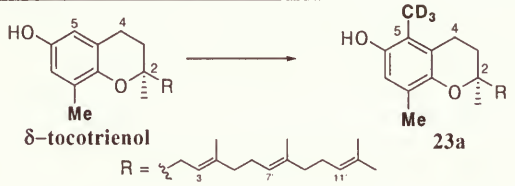
(36578 DPM/ 2 μL) x (25 x 10^3 μL/2 μL) / (2.22 x 10^{12} DPM/Ci) = 205.7 μCi

205.7μCi in 12.4 mg of radiolabelled 5-^{14}C-methyl-α-tocotrienol, corresponds to a specific activity of 0.7mCi/mmole. The percentage radioactivity of this product over the starting material $(^{14}CD_2O)_n$ is: 205.7 μCi/ 250 μCi = 82%. This means that 82% radioactivity of the starting material $(^{14}CD_2O)_n$ was retained in the product 5-^{14}C-methyl-α-tocotrienol. But it is impossible to have an 82% radiochemical yield and a 27% chemical yield at the same time. After we contacted the supplier, American Radiolabelled Chemical Inc., the company made a comment about this. Sometimes, the specific activity of they sent to customers is higher than they wrote on the technical data sheet. According to our result, it can be deduced that the specific activity from Radiolabelled Chemical Inc. was 762 μCi instead of 250 μCi.

This is the first synthesis of the ^{14}C labelled vitamin E of its kind, and this would be a highly valuable addition to the biological studies of vitamin E. Table 5 summarizes the deuterium incorporation of each method discussed above, it can be concluded that Method III, **Synthesis via aminomethylation,** is the most optimized one in terms of deuterium incorporation.

Table 5. Deuterium incorporations of tocotrienol analogues.



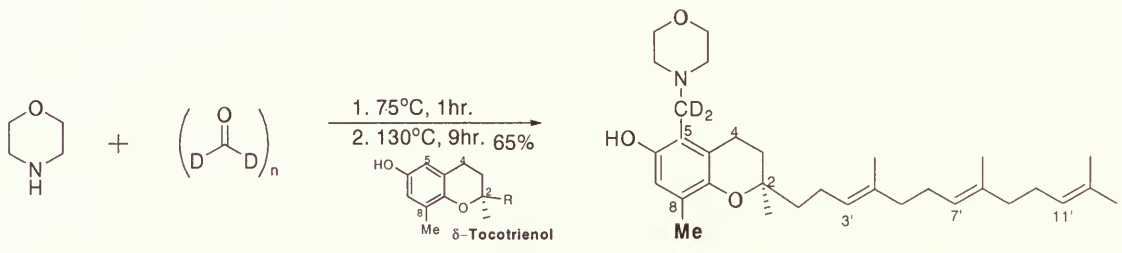
Reaction Conditions	Deuterium Incorporation
Method I, Lewis acid catalyzed method, using $SnCl_2$, $(CD_2O)_n$, DCl, Entry 6 in Table 4.	d_3 92.1%; d_4 3.7%; d_5 4.2%.
Method I, Lewis acid catalyzed method, using $SnCl_2$, $(CD_2O)_n$, DCl, refluxing 92 hours, Entry 7 in Table 4.	Mass of multiple deuterated products can be found in MS.
Method II, Transmetalation Method, see Scheme 10 for detail conditions.	d_3 88.8%, d_4 7.9%, d_5 3.3%.
Method III, Synthesis via aminomethylation using $(CD_2O)_n$, morpholine, then $NaCNBD_3$. See Scheme 8 (a, b) for detailed conditions.	d_3 98%; d_4 1.5%; d_5 0.5%.
	
Method III, Synthesis via amino methylation using $(CD_2O)_n$, morpholine, then $NaCNBD_3$. See Scheme 9 for detailed conditions.	d_3 95.3%; d_4 4.8%; d_5 1.9%.

Furthermore, with the commercially available tritio-*para*-formaldehyde and sodium cyanoborotritide, it is also possible that this methodology can be used in the preparation of tritium labelled α–tocotrienol which would also be useful for biological study of vitamin E, but our collaborator Dr. Chanden Sen specifically requested ^{14}C-labelled material.

2.2 Regioselective Synthesis of 5-Trideuteromethyl-β-tocotrienol

γ-d_3-Tocotrienol was our next synthetic target because of interest in its biological activity [34]. With the expectation that 7-deuteromethyl-d_6-γ-tocotrienol could also be prepared using the Mannich reagent, an attempt was made starting with δ-tocotrienol under the same reaction conditions as above. Since in δ-tocotrienol, there are the two available *ortho* positions next to the hydroxyl group in the chroman moiety, it is possible to form di- and/or mono-methylated products. Unfortunately, the regioselectivity was not in our favour, since the monomethylation of δ-tocotrienol gave **23a**, 5-trideuteromethyl-β-tocotrienol (Scheme 14). The structure of **23a** was confirmed on the basis of NOE effects. Irradiation of the C-8 methyl proton at δ =2.12 ppm gave a large NOE effect for the aromatic proton resonance at δ =6.50 ppm. This indicated that this aromatic proton must be attached to C-7, not C-5; thus the trideuteromethyl group must be at C-5 to give compound **23a** as β-tocotrienol, not compound **23b** as γ-tocotrienol. Therefore, we concluded that the regioselectivity of this method makes it impossible to prepare γ-d_3-tocotrienol, but only gave the β-d_3-tocotrienol which is of little interest for biological studies.

Scheme 14. Synthesis of 5-CD_3-β-tocotrienol



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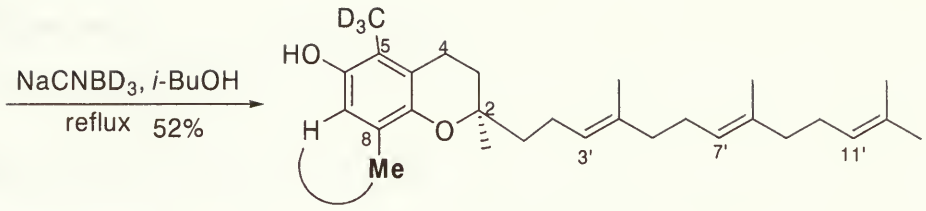
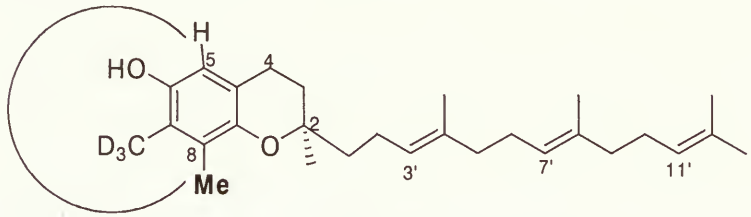


large nOe

23a. 5-CD_3-β-tocotrienol



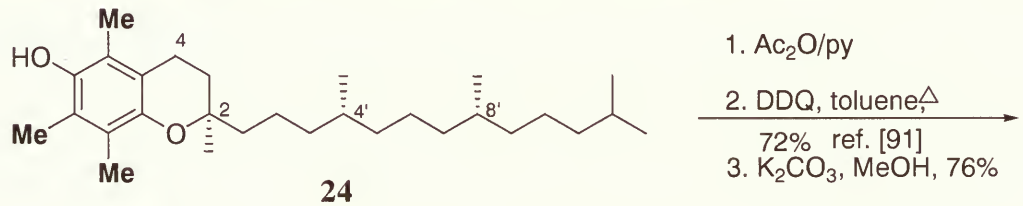
no nOe

23b. 5-CD_3-γ-tocotrienol

2.3 Preparation of 3, 4-D_2-α-tocotrienol

3,4-D_2-α-tocotrienol with two deuterium atoms in the nonaromatic ring of the chroman head would be a useful analog of deuterium labeled α-tocotrienol in bioactivity studies. The corresponding analog of α-tocopherol had been synthesized by Ingold and his coworkers [70]. Recently our research group used an intermediate chromene, in the synthesis of photoaffinity label analogs of α-tocopherol [91]. As shown in Scheme 15, the C3-C4 double bond in the nonaromatic ring of the chroman moiety was installed by DDQ oxidation of α-tocopherol [91]. The subsequent Bouveault-Blanc reduction of [3, 4-dehydro]-2*R*, 4'*R*, 8'*R*-α-tocopherol, **25**, with Na in C_2H_5OD [32] provided compound **26** with two deuterium atoms in the molecule.

Scheme 15. Synthesis of [3, 4-didutero]-2*R*, 4'*R*, 8'*R*-α-tocopherol



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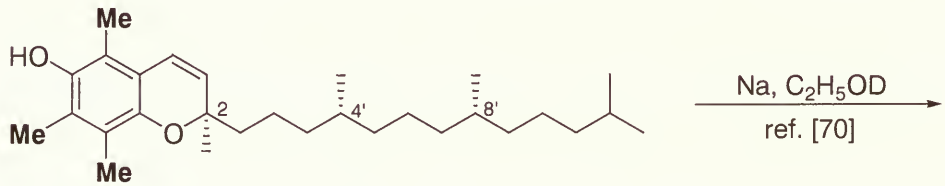

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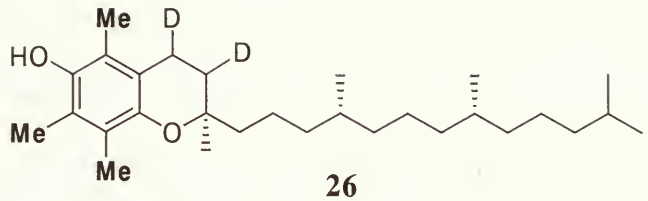


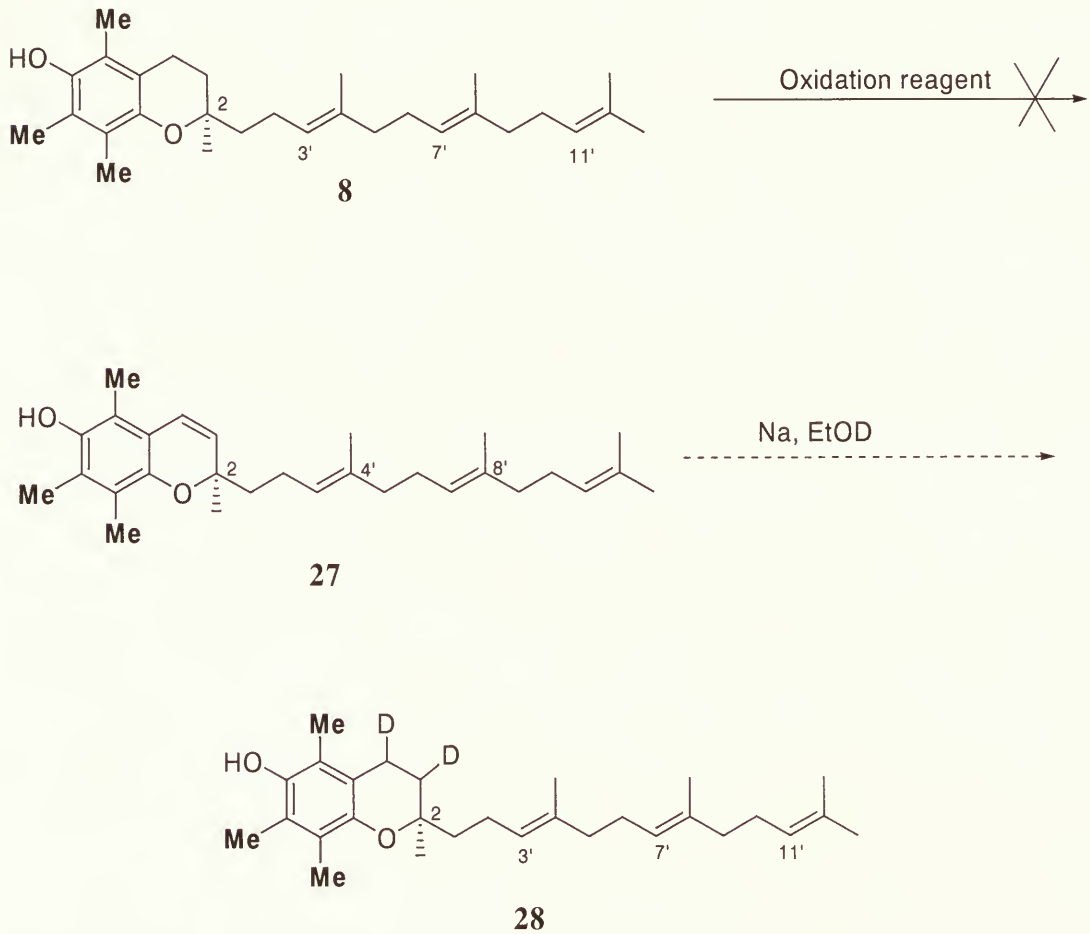
26

Both of these steps proceeded readily. In the first step, the oxidation of α-tocopherol acetate went smoothly with 72% yield [91] by simply refluxing tocopherol in toluene in the presence of DDQ for overnight; the later deuteration step gave product with 100% d_2 incorporation in 70% yield [32] by refluxing the mixture of compound **25** and sodium metal in EtOD for 1.5 hour then left stirring at room temperature for further 24 hours. Our research group also reported the hydrogen-deuterium exchange during the reductive deuteration of α- and γ-tocopherol chromenes [33]. It was found that the reduction of chromenes with heterogeneous catalysts and deuterium gas resulted in various degrees of deuterium incorporation even though high purity deuterium gas was used. It has proven to be the exchange of deuterium with the hydrogen on the C-7 of the γ-tocopherol chromenes. This could be controlled to give 94% d_2 incorporation using 10% Pd/C at 0°C in ethyl acetate.

Hoping that the above method using DDQ/ Na-C_2H_5OD could be applied to α- and/or γ-tocotrienol and give 3, 4-D_2-α- and/or γ-tocotrienol respectively, we performed exactly the same reaction sequences on the tocotrienol substrates. However, surprisingly and disappointedly, we found that the desired oxidation reaction did not occur with the tocotrienols no matter how we adjusted the

reaction conditions including refluxing the reaction mixture in toluene or xylene in a flask or heating it in a sealed high pressure tube for various lengths of time from 2 to 36 hours, or even with running the reaction in a microwave oven in methanol (Scheme 16). Either starting materials were recovered or uncharacterizable products resulted.

Scheme 16. Proposal of synthesis of [3, 4-didutero]-α-tocotrienol



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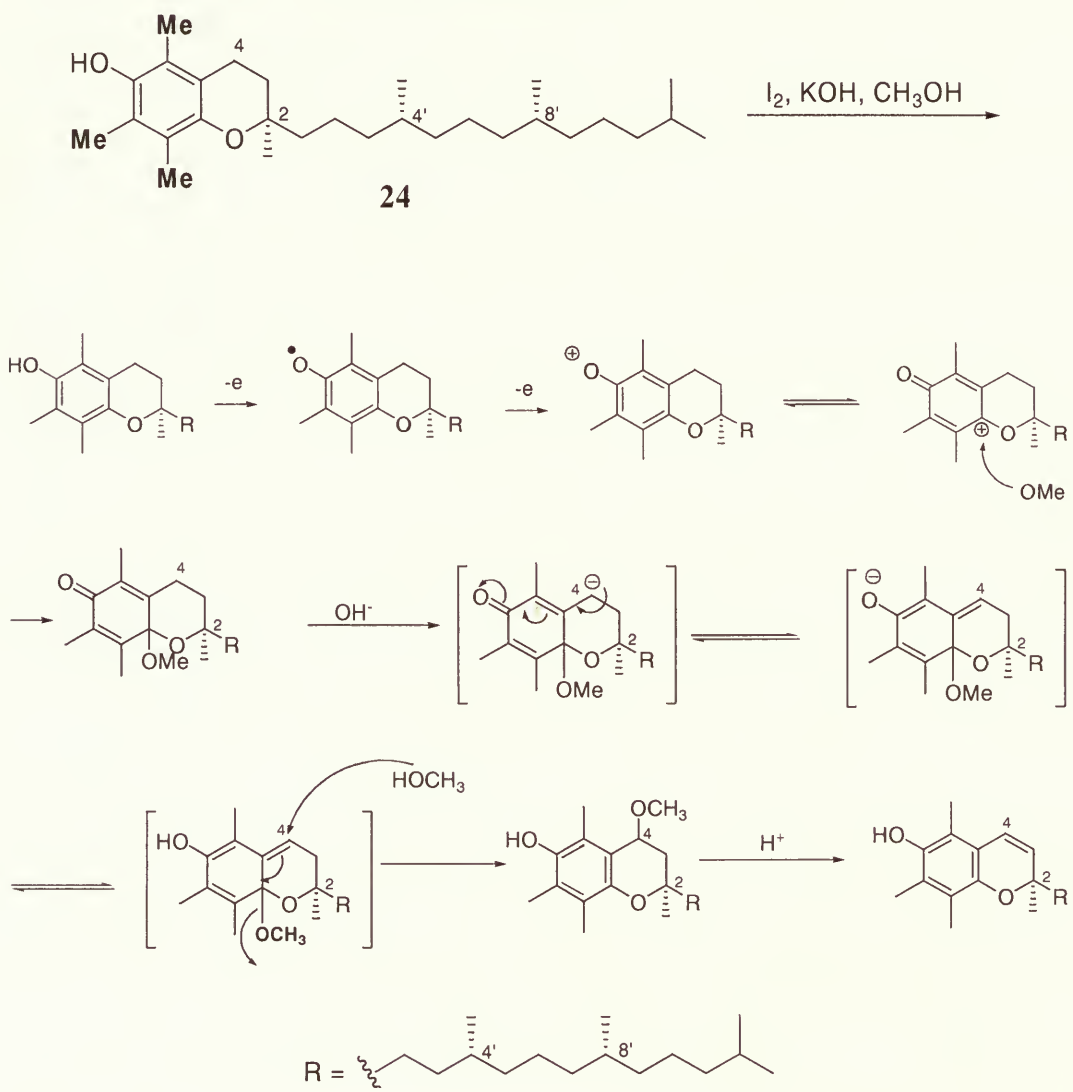
It has been reported that a one–pot alkaline iodine oxidation of α-tocopherol [92], followed by heating and acidification with hydrochloric acid gave chromene in 69% yield (Scheme 17).

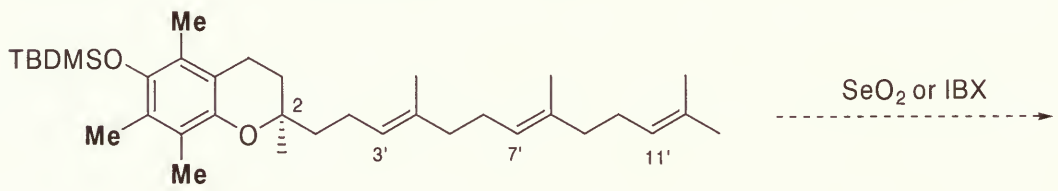
Scheme 17. Iodine oxidation of α-tocopherol



Although this method works well with α-tocopherol, not even a trace of the desired product was produced when used with α-tocotrienol.

SeO_2 [84] and IBX [94] had also been used as oxidation reagents to give benzylic alcohols or benzylic ketones. Scheme 18 shows another strategy designed to install the C3-C4 double bond. It was hoped that the SeO_2 or IBX oxidation of the α-tocopherol TBDMS ether would give either the benzylic alcohol at C-4 or benzylic ketone which could be reduced to alcohol, the dehydration of alcohol would install the double bond between C3 and C4, then the 3,4-dideutero atoms could be introduced by known method [70]. Unfortunately, however, none of these oxidation reagents afforded desired product, benzylic ketone **30** or benzylic alcohol **31**. Only a mixture of several byproducts was obtained, and it can be observed from the ^{1}H-NMR spectrum of the crude product that the vinyl proton resonances at δ=5.2 ppm vanished. Obviously, no desired product with the double bond at C3 and C4 position was formed under these oxidation conditions.

Scheme 18. Proposal of synthesis of compound 33 by using SeO_2/IBX



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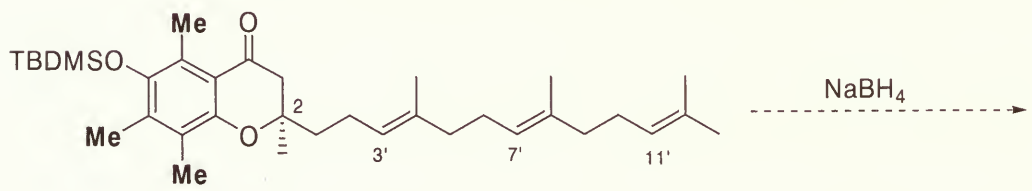

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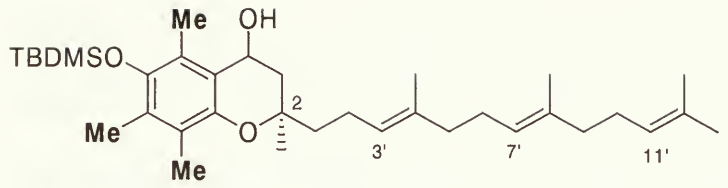

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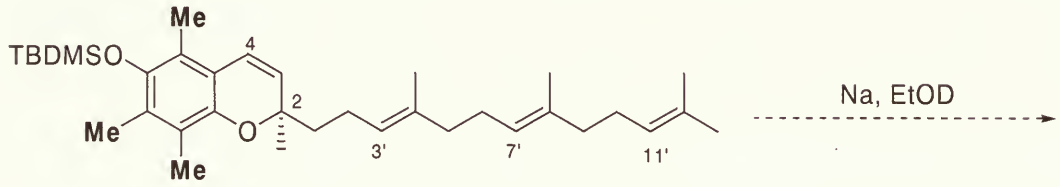

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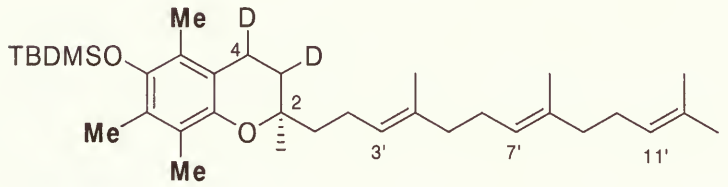


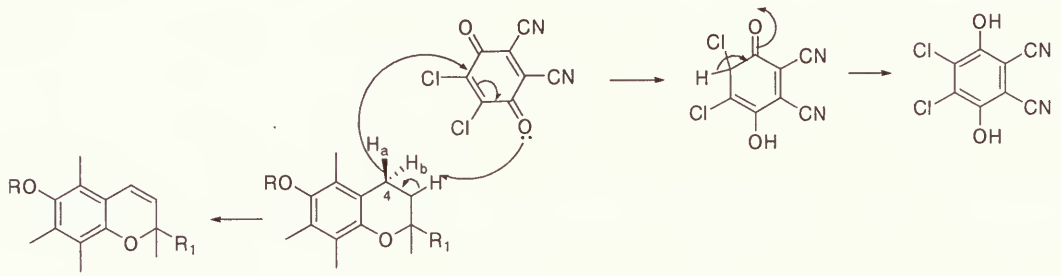
33

It is odd that the DDQ oxidation of α-tocopherol should work so well, and α-tocotrienol fail so completely given the high degree of structural similarity between the two molecules. It became apparent that there must be some unusual structural features of the phytyl side chain. A reasonable explanation was proposed through a further investigation of the conformational features of tocopherol and tocotrienol via computational chemistry. To elucidate the factors responsible for the differences in reactivity between α-tocophenol and α-tocotrienol, we got great help from Dr. Travis Dudding (Department of Chemistry, Brock University, St. Catharines) and a series of computational calculations were performed utilizing molecular mechanics. To ensure an

adequate sampling of the active conformation space available to the two tocopherols, the use of Monte Carlo Multiple Minimum (MCMM) conformational searches was deemed optimal. All of the reported simulations were performed with the MMFFs force field as implemented in Macromodel 8.6[i] using the default settings of the program.

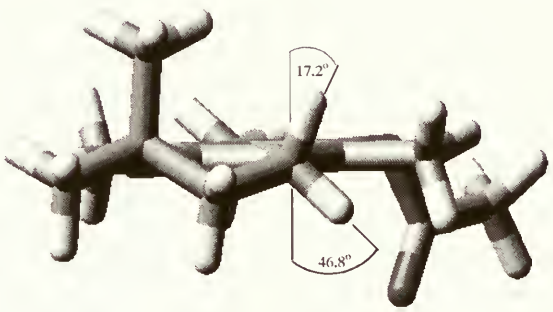
From a mechanistic standpoint, oxidation of the chromanol ring presumably involves the selective homolytic C-H fragmentation of the axial benzylic hydrogen which is optimally positioned for delocalization of the developing carbon center radical into the neighboring aromatic π-system during bond cleavage (Scheme 19).

Scheme 19. Mechanism of DDQ oxidation of vitamin E



The corresponding torsion angles for $\sigma_{C\text{-}H} \rightarrow \pi_{Ar}$ orbital overlap for a simplified chromanol ring system (calculated at the B3LYP/6-31G(d) level of theory) are consistent with this hypothesis judging from measured axial (dihedral = 17.2°) and equatorial (dihedral = 46.8°) dihedrals between the benzylic hydrogens and aromatic π-system, Figure 7. The pseudo-axial proton H_a at C-4 is more likely to be removed than H_b since the resulting cation will be stabilized by the π-electron system of the aromatic ring [95].

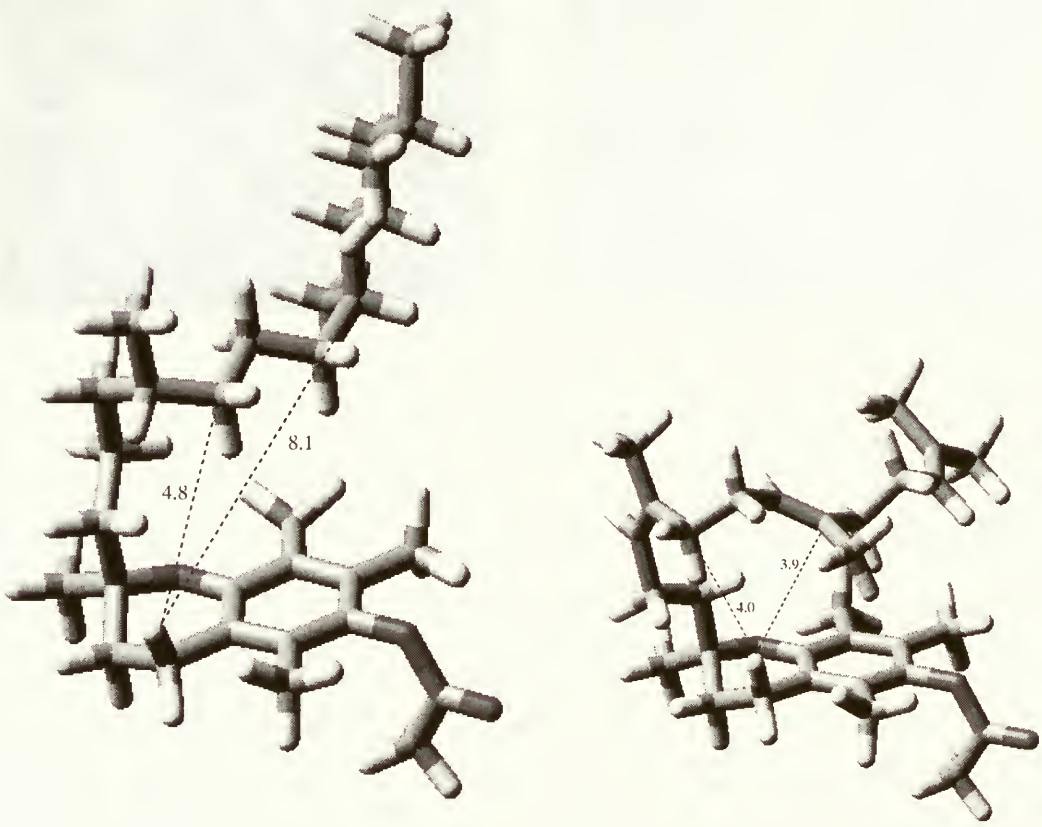
Figure 7. Measured dihedral angles of the axial and equatorial benzylic hydrogens with respect to the aromatic π-system.



Based on this mechanistic consideration, inspection of the low energy α-tocophenol and α-tocotrienol structures (**a**) and (**b**) depicted in Figures 8 provided insight into the differential reactivity of the two systems. Figure 8 (**c**) and (**d**) show a selection of confirmations of α-tocopherol and α-tocotrienol respectively, from which the lowest energy confirmations (**a**) and (**b**) are deduced according to their highest appearance frequency. The most notable feature of the two is placement of the lipid tail atop the chromanol ring system. For illustrative purposes, select contacts between the benzylic hydrogen and proximal atoms of the α-tocophenol and α-tocotrienol side chains are highlighted (4.8 Å *vs.* 4.0 Å, 8.1 Å *vs.* 3.9 Å). In this regard, the comparatively shorter distances found between the axial benzylic hydrogen and lipid side chain in α-tocotrienol results in more effective shielding of this center and is the decisive factor responsible for α-tocotrienol's lack of chemical reactivity.

Figure 8. Select benzylic-H•••C distances for structures of α-tocopherol acetate and α-tocotrienol acetate.

a. α-tocopherol acetate **b. α-tocotrienol acetate**



c. Multiple confirmation of α-tocopherol

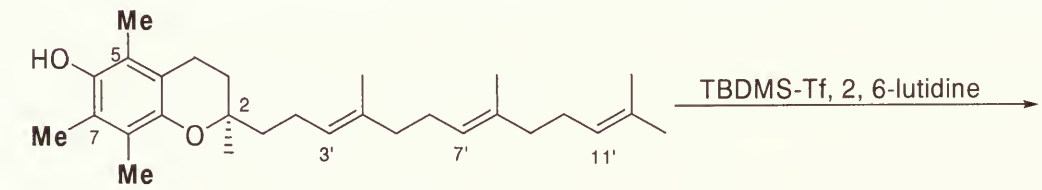
d. Multiple confirmation of α-tocotrienol

2.4 Deuteration of α-tocotrienol silyl ether

The preparation of 5-trideuteromethyl-α-tocotrienol **1,** would also be accomplished if the methyl groups at C-5, C-7 and/or C-8 could be deprotonated in the presence of base, and then deuterated upon trapping with D_2O. A similar method had been used to generate the benzylic anion followed by its deuteration in the investigation of nature product total synthesis [96].

The free phenolic hydroxyl group of α-tocotrienol has to be protected before the treatment with base (Scheme 20). In the presence of 2,6-lutidine, the C-6 hydroxyl group of chroman was readily protected as its *tert*-butyldimethyl silyl (TBDMS) ether. Both *t*-BuLi and *n*-BuLi were used in different molar equivalents under either kinetic or thermodynamic deprotonation conditions at low or moderate temperatures. The reaction mixture was then quenched with D_2O for deuteration.

Scheme 20. Deuteration test of α-tocotrienol



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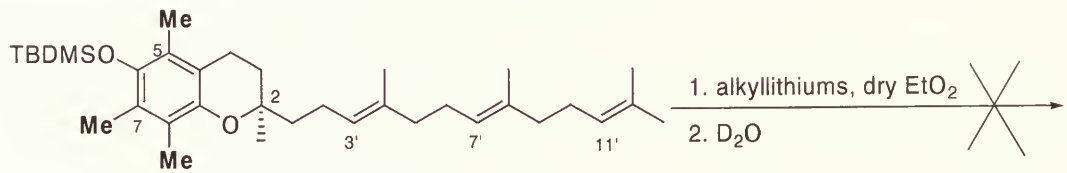

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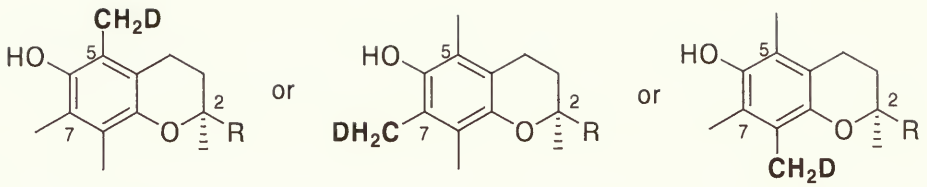
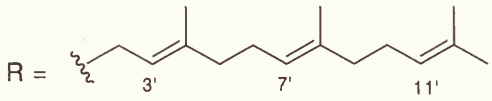


34 **35** **36**

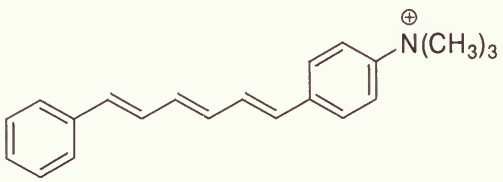


However, disappointingly we found that none of these attempts provide even a trace of deuterated α-tocotrienol when inspected by ^{1}H-NMR and MS. The reason for such failure is likely in the fact that the methyl protons on the benzyl methyls of the chroman have too low acidity (pKa ≈ 41 is for Ar-H) [97] towards even very strong bases. In addition, unlike in other literature where the formed benzylic anion could be stabilized through resonance [96]; in our case, even if the anion did form, the lack of an anion stabilizing functionality on the aromatic ring appears to make it impossible to form the benzyl anion(s) under these conditions. Moreover, the high steric hindrance inherent in the structure due to both the bulky silyl ether protection group, and long phytyl group attached as side chain to the chroman head, may restrict deprotonation. Changing the protecting group to methoxymethyl (MOM) group could be a possible alternative if this reaction would be attempted in the future by other researchers in our academic group.

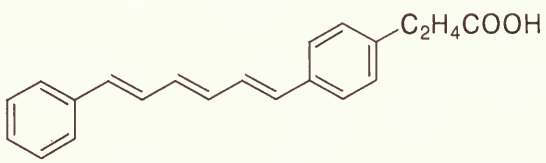
2.5 Attempted preparation of hexaene analog of α-tocotrienol

Conjugated polyenes have been synthesized and utilized as powerful tools in studies on the dynamic nature of bilayer membranes [98]. Generally, these compounds have both a polar moiety (e.g. carboxylic acid group) and a hydrophobic moiety consisting of multi conjugated double bonds, which allows fluorescence spectroscopy to be used to follow the probe as a reporter of membrane fluidity. Some structures of this type of compounds are illustrated in Figure 9 [98].

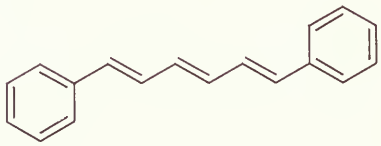
Figure 9. Structure of Poly-ene membrane probes



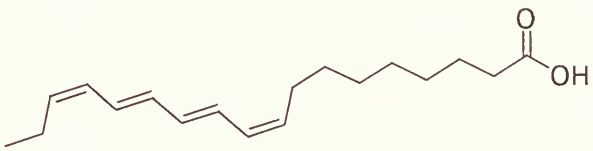
TMA- DPH



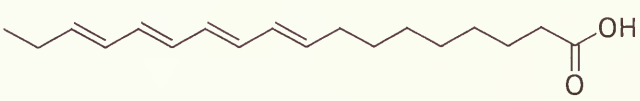
CE-DPH



DPH



***cis*-PA**

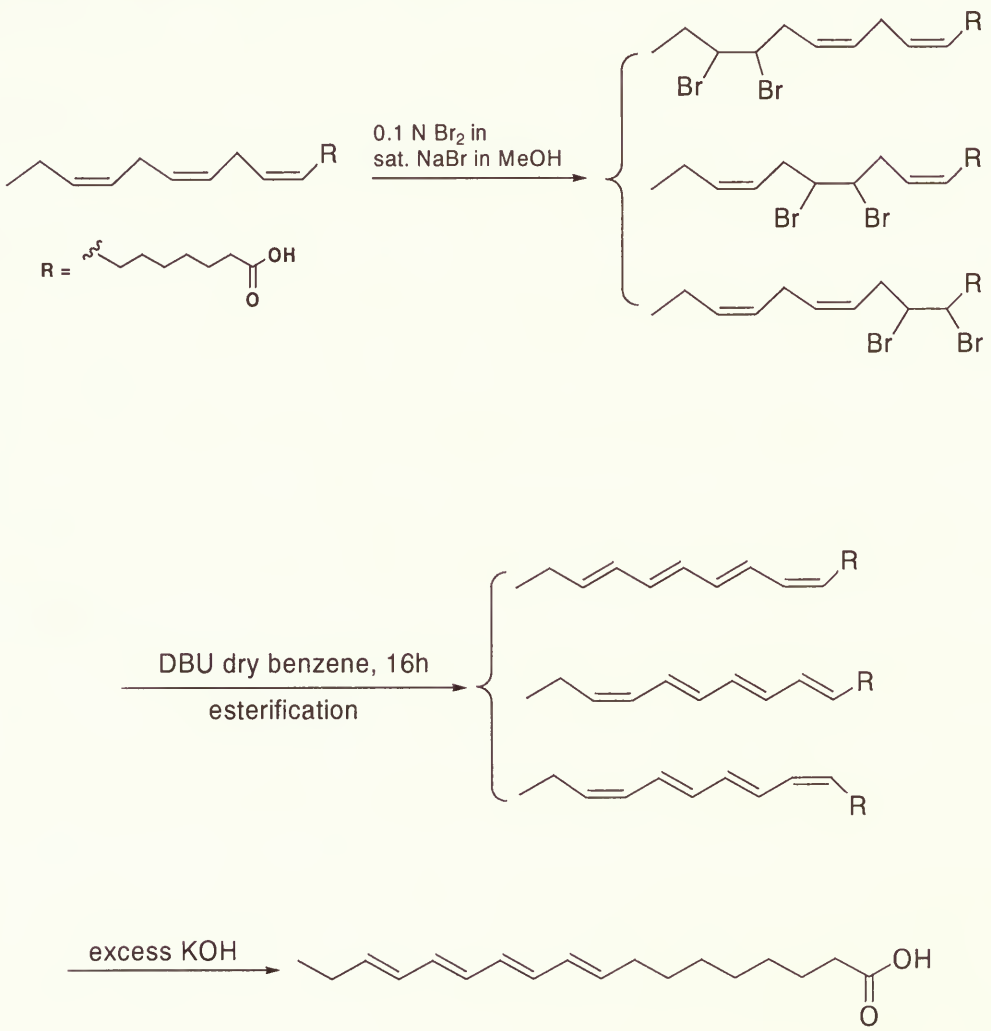


***trans*-PA**

DPH—1,6-diphenyl-1,3,5-hexatriene; CE—2-carboxyethyl; TMA—4-trimethylamino; PA—parinaric acid

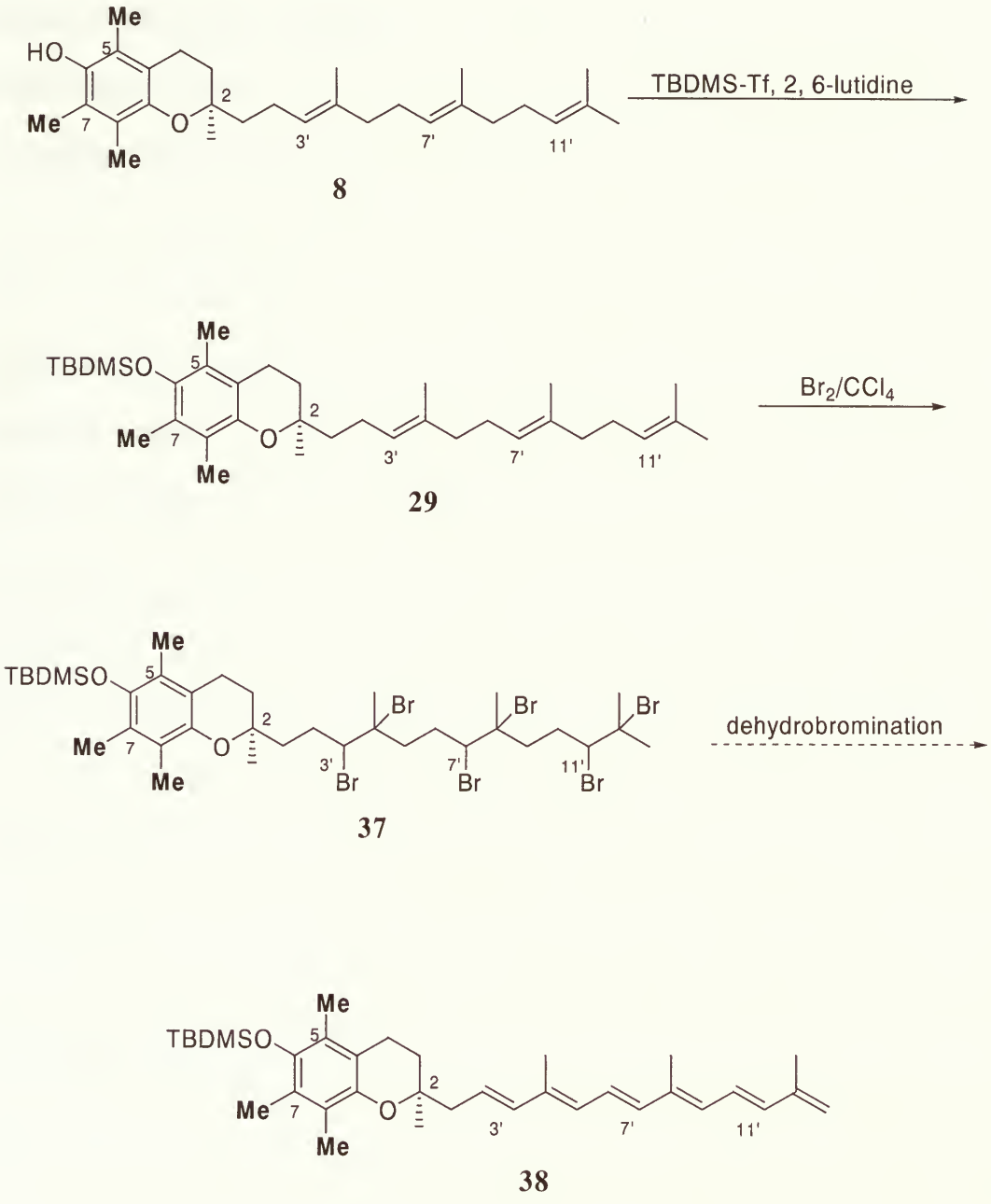
Kuklev *et. al.* reported the synthesis of parinaric acid and its isomers recently (Scheme 21) [99]. Bromination of the methylene-interrupted, *cis* triene system (1,4,7-octatriene) of α–linolenic acid using Br_2 in a saturated solution of NaBr in methanol, followed by the esterification of the fatty acid dibromides and double dehydrobromination by 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) provided the mixture of parinaric acids, which can be converted by base catalyzed cis-trans isomerization to exclusively all-trans conjugated β-parinaric acid.

Scheme 21. Synthesis of *trans*-polyene fatty acid



A similar strategy might be applicable to α-tocotrienol. Complete bromination of the three double bonds at C-3', C-7' and C-11' position in the phytyl group would produce a hexabromo intermediate which might afford *trans* conjugated multiene upon dehydrobromination. With these expectations, the free phenol group of α-tocotrienol **8** was first protected as its TBDMS ether in the presence of a mild base (Scheme 22). However, the subsequent bromination using Br_2 in a saturated solution of NaBr in methanol did not produce even a trace of desired product. The use of $PyH^+Br_3^-$ showed no improvement. Instead, the reaction of **29** with Br_2 dissolved in CCl_4 afforded the hexabromo compound **37** as yellow oil in good yield with correct molecular ion mass and isotope pattern (M^+ = 1018, 100%; 1016, 73%; 1017, 31%; 1019, 42%; 1020, 79%) in the mass spectrum. The next step is the installation of six conjugated *trans* double bonds through base induced dehydrobromination. Treatment by DBU produced no desired dehydrobromination product **38**, unlike the results reported by Kuklev [99]. Several products resulted from the DBU treatment of the hexabromide and that while some bromines appear to have been removed, no specific products could be characterized. Various kinds of bases in different solvents were used for this dehydrobromination step, such as sodium hydride or potassium *t*-butoxide in dry tetrahydrofuran (THF), *n*-butyllithium in dry ether, sodium hydroxide in ethanol, and sodium ethoxide in EtOH or silver oxide in ethyl acetate. Unfortunately, none of these conditions gave the desired product **38**; in most cases, only multiple products were observed according to thin layer chromatography (TLC).

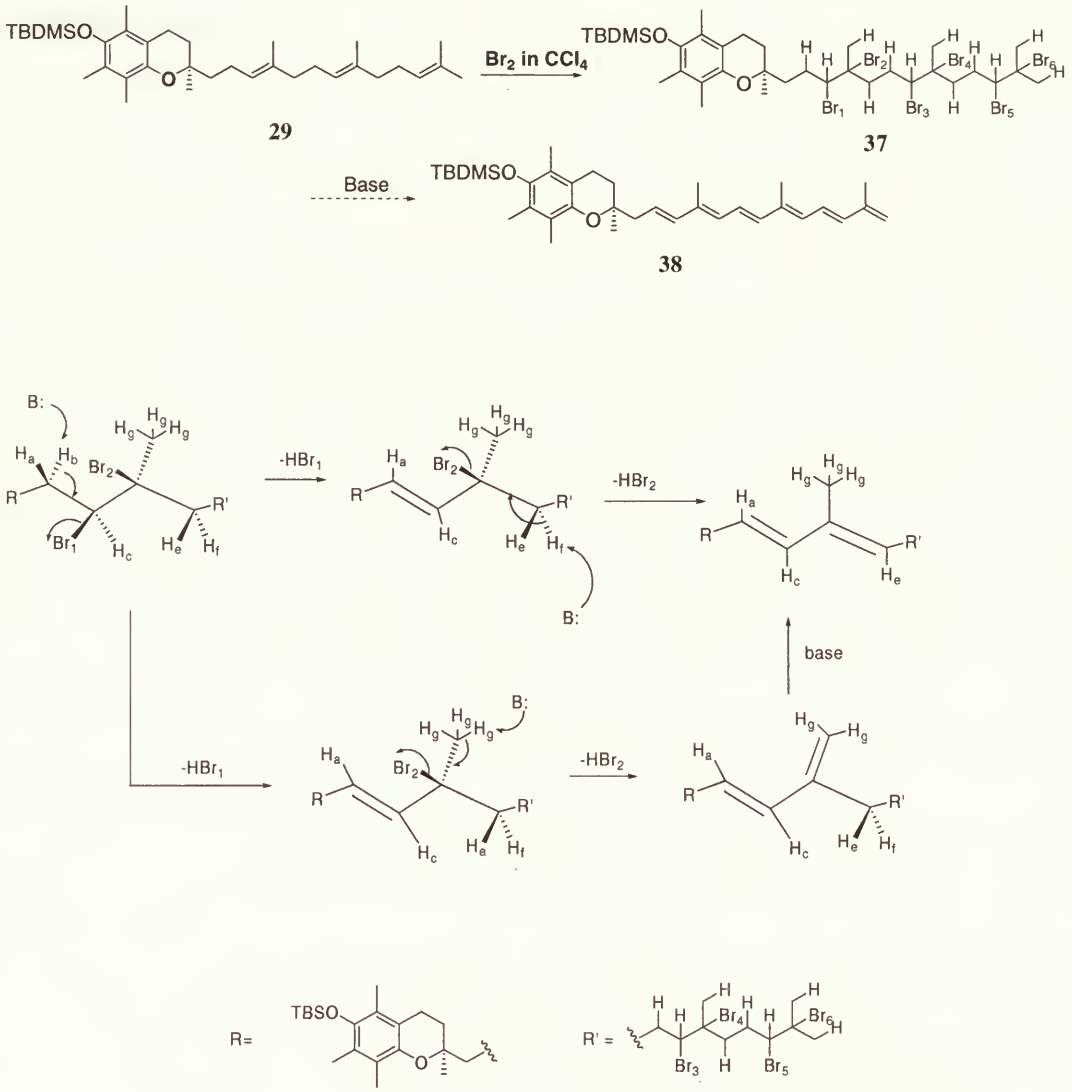
Scheme 22. Synthesis of *trans*-hexaene tocotrienol analogue via dehydrobromonation approach



During our exploration of the reason for this unsuccessful dehydrobromination, we realized that the difficulty of this reaction might lie in the structural nature of the six bromo substituted phytyl group. It is known that in a E2 reaction, the

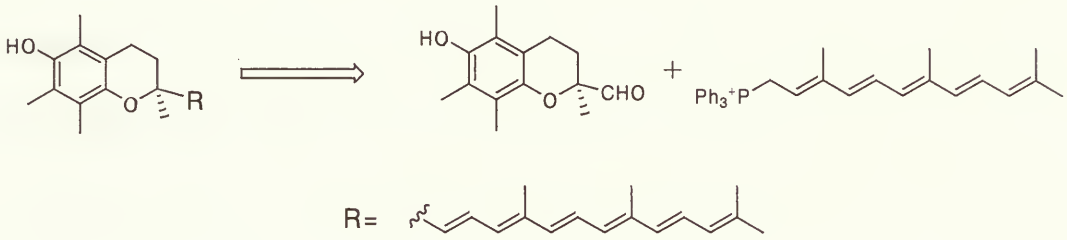
attacking base begins to abstract a proton from a carbon next to the leaving group, the C-H bond begins to break , a new C=C bond begins to form and the leaving group begins to depart, taking with it the electron pair from the C-X bond. The elimination works best when a proton is at an anti periplanar geometry position to its adjacent living group. The same principal applies here in our case. Scheme 23 shows the pathway of elimination of dibromo segments to yield two C=C bond. Br_1 can only be eliminated along with H_b which is the only anti periplanar proton available for it. Once the first C=C bond was formed, the second C=C has two possible geometries, of which the exo-methylene one can be isomerized to a *trans* double in the presence of base. If all the six bromines in the molecule were to be eliminated in this ideal two-by-two pattern, the target compound would be formed with six conjugated double bonds as we expected. However, the problem here is that due to the number of bromines presented, there is a high unpredictability about how many bromines could be eliminated under the reaction condition, and/or in what pattern these bromines would be lost. It is possible that all of the eliminations pattern would possibly occur at the same time and give a mixture of mono-, di- and multi-dehydrobromination products, which is consist with the actual experimental observation of streaking, not clearly separated spots on TLC and uninterpretable resonance in ^{1}H-NMR spectrum of crude material.

Scheme 23. Dehydrobromination mechanism of compound 37

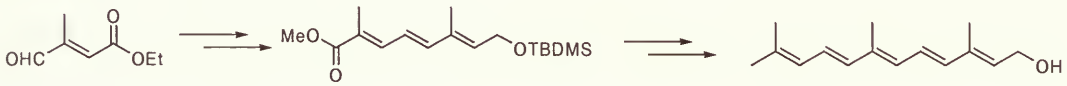


The unpromising results of dehydrobromination force us to seek alternative routes to synthesize the hexaene **38**. Scheme 21b shows the idea of preparation of this type of compound through the Wittig olefination between the chroman aldehyde and a polyene ylide. Some other research groups had reported the synthesis of the polyene ylides as briefly shown in the same scheme (Scheme 24) [100].

Scheme 24. Synthesis of *trans*-hexaene tocotrienol analogue via Wittig olefination approach



Ref. [99]



Once the Wittig olefination was achieved, it would be the first time for compound **38** to be synthesized of its kind. After deprotection, compound **38** might become a powerful tool as a novel fluorescent probe for the bioactivity analysis of tocotrienol, as fluorescent peaks are expected at λ_{abs} ~360nm and λ_{em} ~ 465nm..

3. Summary and Future Work

In summary, the synthetic work of this thesis highlighted the methodology for the first synthesis of d_x-deuterated (x > 1) tocotrienols with satisfactory yield using naturally occurring starting material. This methodology provided a convenient route for multi-gram preparation of d_3-deuteromethyl α-tocotrienol which can be used as isotopic tracers for studies of tocotrienols. In addition, similar method was used to afford 5-^{14}C-methyl α-tocotrienol. This is the first synthesis of the ^{14}C labeled tocotrienol is a highly valuable addition to the biological studies of vitamin E. Furthermore, preliminary attempts on the synthesis of polyunsaturated tocopherols with a hexaene functionality in the phytyl side chain as a new fluorescent probe have been conducted and discussed in this thesis.

4. Experiments

4.1 General

All reagents were purchased from commercial suppliers (Aldrich Chemical Company, Oakville, ON; CDN Isotopes. Inc. Pointe-Claire, QC; Carotech Sdn Bhd. Malaysia for TOCOMIN 50%®, and American Radiolabelled Chemical Inc.). Reactions that were expected to be sensitive to air or moisture were performed under an inert atmosphere of argon. All glassware and syringes were dried in an oven 60°C-80°C, and then cooled in a dry box before use. All temperatures are in °C. Low temperature baths were prepared with acetone/liquid N_2 for -78°C, ethanol/ liquid N_2 for -20°C and ice/ water for 0°C. A constant temperature silicon oil bath was used for heating reaction mixtures at temperature above room temperature. Air sensitive reagents and solutions were transferred via syringes and were introduced under argon. Removal of solvent was normally accomplished using a reduced pressure rotary evaporator (10-15 mmHg) and vacuum pump (0.3-0.5mmHg).

Reagent-grade solvents were used for all extractions and work-up procedures. Distilled water was used for all aqueous workups and all aqueous solutions. Tetrahydrofuran (THF) and benzene were distilled from sodium benzophenone ketyl. Dichloromethane, hexane, trimethylsilyl chloride (TMSCl) and diethyl ether were distilled from CaH_2. Dry methanol and ethanol were distilled from magnesium and catalytic amount of iodine.

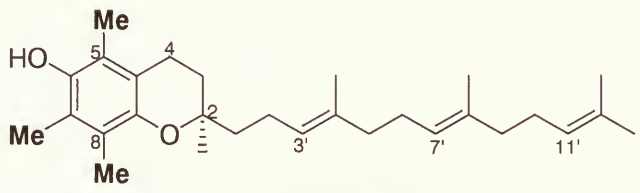
Thin layer chromatography (TLC) was carried out on Merck pre-coated silica gel 60 F_{254}, aluminium sheets, 200μm thick, 25mm (width) x 50mm (length). After development, the sheets were viewed under short and/or long wavelength UV light or with an oxidizing staining solution consisting of 4 % sulphuric acid in methanol, followed by heating using hot air gun. Flash chromatography was performed using Merck 9385 silica gel 60 (230-400 mesh). Fourier transform infrared spectra (FT-IR) were recorded on a Bomem MB-100 spectrometer as neat films between NaCl plates, or as KBr discs. Low resolution mass spectra (MS) were recorded on a Carlo Erba/ Kratos GC/MS Concept 1S double focusing mass spectrometer interfaced to a Kratos DART acquisition system and a Sun SPARC workstation. Samples were introduced through a direct inlet system. Ions were generated using electron impact (EI) at 70ev or Fast Atom Bombardment (FAB) sources and were reported as m/z values for the parent peak and major fragments. ^{1}H (300 MHz) and ^{13}C NMR (75 MHz) spectra were obtained on Bruker DPX-300 digital FT NMR spectrometer with deuterated chloroform as solvent unless otherwise stated. Chemical shifts for NMR were determined relative to the internal standard tetramethylsilane (δ 0.00 ppm) or $CHCl_3$ (δ 7.24 ppm) for ^{1}H spectra, and $CDCl_3$ (δ 77.0 ppm) for ^{13}C spectra. All ^{1}H-NMR data listed have the following order: chemical shift (ppm), (multiplicity, number of protons, coupling constants, assignment). Multiplicity is designed using following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). The ^{13}C-NMR data listed in chemical shift (ppm). The

numbering systems for all the polycyclic compounds were assigned according to the Chemical Abstract Service's ring index.

4.2 Separation and Purification of Tocotrienols from Commercial Palm Oil Suspension TOCOMIN®50%

TOCOMIN®50% contains 50% (w/w) natural full spectrum tocotrienol/tocopherol complex, which consists of predominantly α-, β-, γ-, δ-tocotrienol and α-tocopherol. Column chromatography was used to separate and purify these ingredients for future usage as starting materials in the syntheses of deuterated tocotrienols. After several TLC attempts, 10% EtOAc in hexane was chosen as the optimum solvent system to conduct the separation. 320mL silica gel and 2L solvent (10% EtOAc in hexane) were used on 2.02g TOCOMIN®. The purification result is listed in Table 3.

4.2.1 α-tocotrienol



α–Tocotrienol

TLC R_f=0.45 (hexane/EtOAc=9:1)

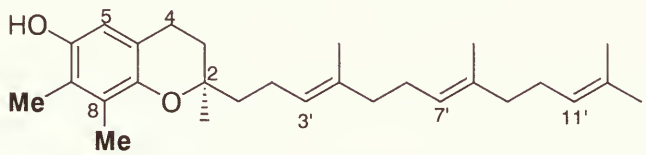
^{1}H-NMR ($CDCl_3$) 5.12(m, 3H, C3'-H, C7'-H, C11'-H), 4.21(s, 1H, OH), 2.63(t, 2H, *J*=7Hz, C4-CH_2), 2.17(s, 3H, Ar- CH_3), 2.13(s, 3H, Ar-CH_3), 2.13(m, 2H, C2'-CH_2), 2.12(s, 3H, Ar- CH_3), 2.10(m, 4H CH_2), 1.99(m, 4H, CH_2), 1.80(m, 2H, C3-CH_2), 1.68(s, 3H, CH_3), 1.65,1.55(m, 1H of each, C1'-CH_2), 1.62(m, 6H, C12'-2CH_3), 1.60(s, 3H, CH_3), 1.26(s, 3H, C2-CH_3).

^{13}C-NMR ($CDCl_3$) 145.63, 144.71, 135.17, 135.08, 131.37, 124.55, 124.35, 122.76, 121.18, 118.63, 117.43, 74.42, 39.85, 39.67, 31.72, 26.90, 26.74, 25.83, 23.86, 22.37, 20.88, 17.82, 16.13, 16.03, 12.34, 11.91, 11.40.

MS[EI+](%) m/z 424(M+, 71.1), 205(15.1), 165(100), 69(47.9).

HRMS 424.33455

4.2.2 γ--tocotrienol



γ–Tocotrienol

TLC R_f=0.32 (hexane/EtOAc=9:1)

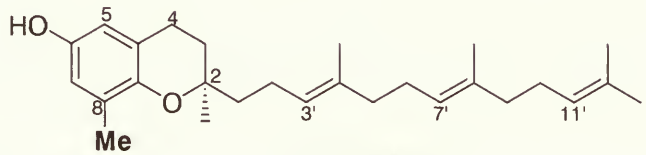
^{1}H-NMR ($CDCl_3$) 6.37(s, 1H,Ar-H), 5.12(m,3H,C3'H, C7'H, C11'H), 4.46(br, 1H, OH), 2.68(m, 2H, C4-CH_2), 2.18(m, 2H, C2'-CH_2), 2.15(s, 3H, Ar-CH_3), 2.13(s, 3H, Ar-CH_3), 2.08(m, 4H, 2CH_2), 2.00(m, 4H, 2CH_2), 1.80,1.74(m, 2H, C3-CH_2), 1.69(s, 3H, CH_3), 1.65, 1.58(m, 1H of each, C1'-CH_2), 1.62(s, 6H, C12'-2CH_3), 1.60(s, 3H, CH_3), 1.26(s, 3H, C2-CH_3).

^{13}C-NMR ($CDCl_3$) 146.44, 145.78, 135.19, 135.07, 131.36, 125.91, 124.54, 124.48, 124.33, 121.81, 118.32, 112.29, 75.34, 39.91, 39.84, 31.66, 26.89, 26.72, 25.82, 24.12, 22.41, 22.34, 17.81, 16.12, 16.01, 12.02, 11.99.

MS[EI+](%) m/z 410(M+, 59.8), 191(24.3), 151(100), 69(73.1).

HRMS 410.31697

4.2.3 δ-tocotrienol



δ–Tocotrienol

TLC R_f=0.25 (hexane/EtOAc=9:1)

^{1}H-NMR ($CDCl_3$) 6.33(d, 1H, *J*=3Hz, Ar-H), 6.22(d, 1H, *J*=3Hz, Ar-H), 4.96(m, 3H, C3'-H, C7'-H, C11'-H), 2.53(m, 2H, C4-CH_2), 1.98(s, 3H, Ar-CH_3), 1.87(m, 10H, CH_2), 1.65(m, 2H, C3-CH_2), 1.52(s, 3H, CH_3), 1.42(s, 9H, 3 CH_3), 1.42(m, 2H, C1'-CH_2), 1.11(s, 3H, C2-CH_3).

^{13}C-NMR ($CDCl_3$) 148.18, 146.31, 135.52, 135.36, 131.66, 127.73, 124.79, 124.67, 121.60, 116.03, 112.97, 77.62, 75.69, 40.10, 40.07, 40.06, 30.71, 26.97, 26.85, 26.10, 24.42, 23.06, 22.39, 18.09, 16.46, 16.40, 16.27.

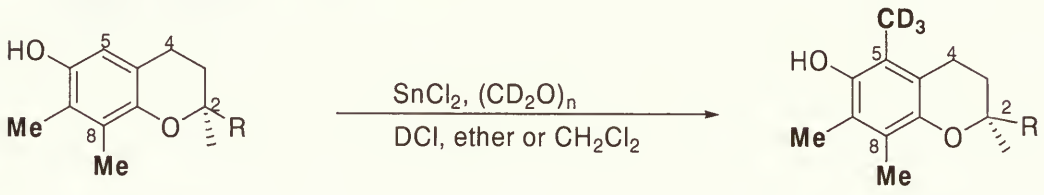
MS[EI+](%) m/z 396(M+, 47.3), 192(13.5), 177(31.4), 137(75.8), 69(100).

HRMS 396.30263

4.3 Synthesis of 5-Trideuteromethyl-α-tocotrienol

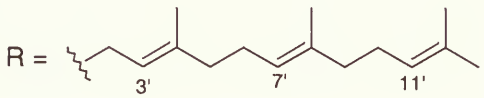
4.3.1 Method I: Lewis acid catalyzed d_3-methylation of γ-tocotrienol.

Reaction Procedures using $SnCl_2$:



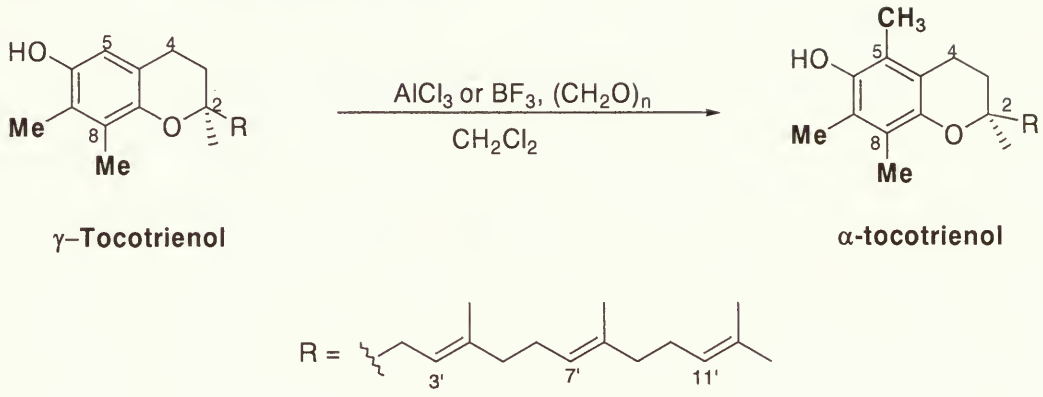
γ–Tocotrienol

5-CD_3-α-tocotrienol



Under argon, γ-tocotrienol (100mg, 24.35mmol) was dissolved in anhydrous isopropyl ether (4 mL) or dichloromethane. Anhydrous stannous chloride was added followed by deuterated hydrogen chloride and paraformaldehyde-d_2. (In some cases, deuterated hydrogen chloride was omitted; see Table 4 for details.) The reaction was then stirred under argon for varying times and at different temperatures. TLC was used to monitor the progress of the reaction (R_f of product=0.45, R_f of substrate=0.32 in EtOAc: hexane =1:9). Ice cold water was added to the reaction mixture and stirred. The aqueous layer was separated and extracted with ethyl ether 3 times. The organic layers were combined and treated with 2 M aqueous Na_2CO_3 solution until pH=7, then washed with water and dried over anhydrous $MgSO_4$. The organic solvent was evaporated *in vacuo*. Silica gel column chromatography using hexane: EtOAc/9:1 was used to purify the product. Yield of products under different reaction conditions are listed in Table 4 (Entry 1-6).

Reaction Procedures using $AlCl_3$ or BF_3:



Under argon, γ-tocotrienol (100mg, 24.35mmol) was dissolved in dichloromethane (2.5mL), $AlCl_3$ or BF_3 was added followed by paraformaldehyde. The reaction was then stirred under argon for varying times and at different temperatures. TLC was used to monitor the progress of the reaction (R_f of product=0.45, R_f of substrate=0.32 in EtOAc: hexane =1:9). Ice cold water was added to the reaction mixture and stirred. The aqueous layer was separated and extracted with CH_2Cl_2 3 times. The organic layers were combined and dried over anhydrous $MgSO_4$. The organic solvent was evaporated *in vacuo*. Silica gel column chromatography using hexane: EtOAc/9:1 was used to give the product as α-tocotrienol. The NMR and MS of this product was identical to that of α-tocotrienol separated from **TOCOMIN®50%.** Yield of products under different reaction conditions are listed in Table 4 (Entry 8-13).

TLC R_f=0.45 (hexane/EtOAc=9:1)

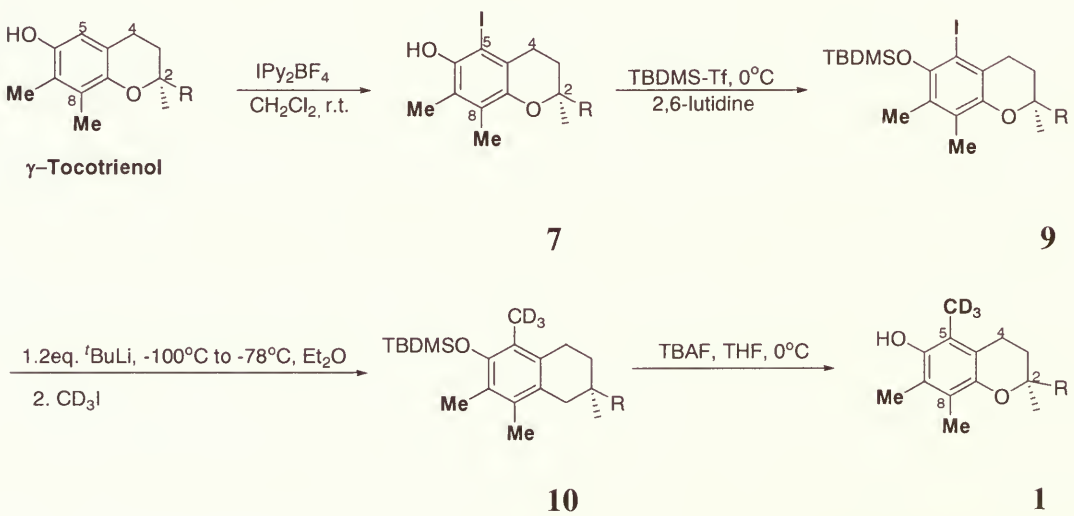
^{1}H-NMR ($CDCl_3$) 5.12(m, 3H, C3'-H, C7'-H, C11'-H), 4.21(s, 1H, OH),

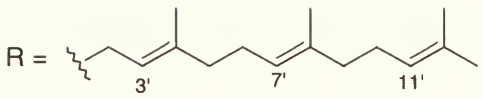
2.63(t, 2H, *J*=7Hz, C4-CH_2), 2.17(s, 3H, Ar- CH_3), 2.13(s, 3H, Ar-CH_3), 2.13(m, 2H, C2'-CH_2), 2.12(s, 3H, Ar- CH_3), 2.10(m, 4H CH_2), 1.99(m, 4H, CH_2), 1.80(m, 2H, C3-CH_2), 1.68(s, 3H, CH_3), 1.65,1.55(m, 1H of each, C1'-CH_2), 1.62(m, 6H, C12'-2CH_3), 1.60(s, 3H, CH_3), 1.26(s, 3H, C2-CH_3).

^{13}C-NMR ($CDCl_3$) 145.63, 144.71, 135.17, 135.08, 131.37, 124.55, 124.35, 122.76, 121.18, 118.63, 117.43, 74.42, 39.85, 39.67, 31.72, 26.90, 26.74, 25.83, 23.86, 22.37, 20.88, 17.82, 16.13, 16.03, 12.34, 11.91, 11.40.

MS[EI+](%) m/z 424(M+, 71.1), 205(15.1), 165(100), 69(47.9).

4.3.2 Method II: Transmetalation strategy.





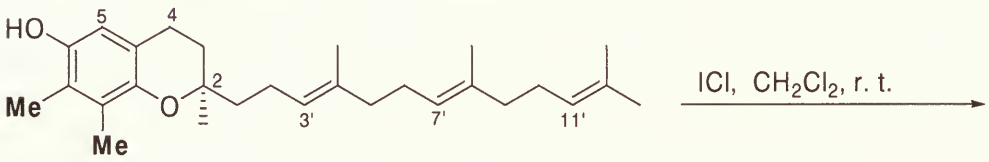
IPy_2BF_4 (128 mg, 0.34 mmol) was added to a solution of γ-tocotrienol (82 mg, 0.20 mmol) in CH_2Cl_2 2.5mL at room temperature under argon. The reaction solution was stirred at room temperature for 1 hour. Water was then added to the reaction and the aqueous layer was separated and extracted with CH_2Cl_2 (3 x 5 mL). Organic layers were combined and dried over anhydrous Na_2SO_4. After filtration, solvent was removed *in vacuo*. Crude product **7** was obtained as dark red oil.

To the cooled (0°C) solution of the above product **7** (252.8 mg) in 2 mL CH_2Cl_2 was added TBDMS-Triflate (56 μL, 0.32 mmol) and 2,6-lutidine (70 μL, 0.6 mmol). The reaction was stirred at 0°C for 1 hour and then quenched with brine. The aqueous layer was extracted with CH_2Cl_2 (3 x 5 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The resulting crude red oil **9** (180.9 mg) was then dissolved in dry Et_2O (2 mL) and cooled to -90°C in a liquid nitrogen/ethanol bath for 15 mins. *t*-BuLi (235 μL, 0.4 mmol) was added to the solution drop wise at -90°C. The reaction was kept stirred at low temperature for 15mins, then CD_3I (13.7 μL, 0.22 mmol) was added and the reaction was allowed to warm to room temperature. Water was added to the reaction mixture and the same aqueous work up as above was applied to provide the crude product **10** as dark red oil.

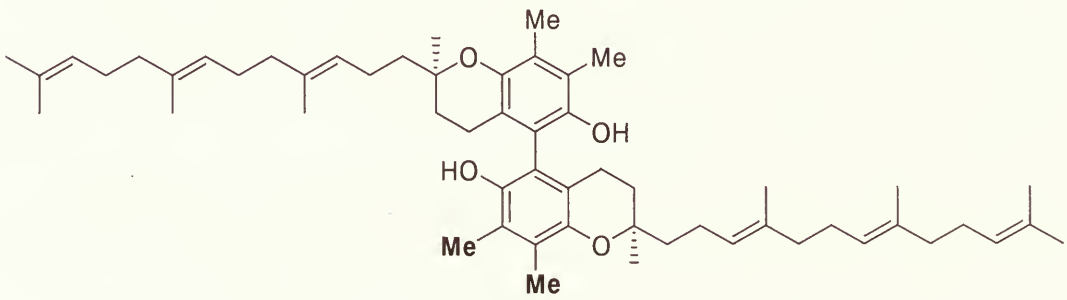
To a solution of crude product **10** (276.9 mg) in dry THF 2mL was charged TBAF (0.4 mL, 0.4 mmol) at 0°C and the reaction mixture stirred at 0°C for 1.5 hours. The reaction was then warmed to room temperature and stirred overnight. Water was added to the reaction mixture followed by the same workup as above to afford crude product which gave 5-trideuteromethyl-α-tocotrienol (9.2 mg, overall yield from γ-tocotrienol 10%) after column chromatography with EtOAc:hexane/1:9.

4.3.3 Formation of dimmer 11

In the attempt to prepare compound 7 using iodochloride, instead of the desired product 7, a dimerization product **11** connecting at C-5 position was formed.



6



11

γ-tocotrienol (50.5mg, 0.12mmol) was dissolved in DCM (3 mL) in a 25 mL round bottom flask. ICl (9μL, 1.78eq) was added to the above solution under argon at room temperature. The reaction mixture was stirred at ambient

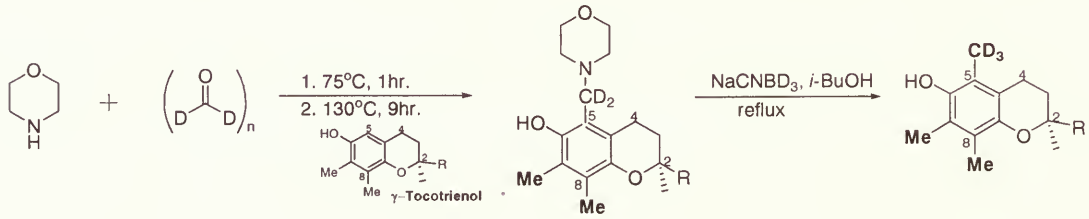
temperature for five days. Aqueous $Na_2S_2O_3$ (5 % w/w) solution was added, organic layer was separated. The aqueous layer was extracted with DCM (3 x 5mL). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated in *vacuo*. Yellow oil was given as crude product, which was purified by flash column chromatography using EtOAc: Hexanes 2% : 98% to give compound **11** as light yellow oil (36.1 mg, 36%).

TLC R_f=0.6 (hexane/EtOAc=9:1)

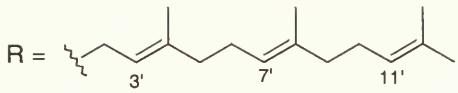
^{1}H-NMR ($CDCl_3$) 5. 20(m, 6H, side chain allylic-H's), 4.50(br, 2H, OH), 2.65(m, 4H, 2C4-CH_2), 2.19(m, 4H, 2C2'-CH_2), 2.17(s, 6H, 2Ar-CH_3), 2.15(s, 6H, 2Ar-CH_3), 2.08(m, 8H, 4CH_2), 2.00(m, 8H, 4CH_2), 1.83,1.77(m, 4H, 2C3-CH_2), 1.72(s, 6H, 2CH_3), 1.69, 1.62(m, 4H, 2C1'-CH_2), 1.63(s, 12H, 2C12'-2CH_3), 1.61(s, 6H, 2CH_3), 1.30(s, 6H, 2C2-CH_3).

MS[EI+] m/z 818 (M+, 5%), 410(45%), 191, (66), 151(100%).

4.3.4 Method III: Synthesis via aminomethylation

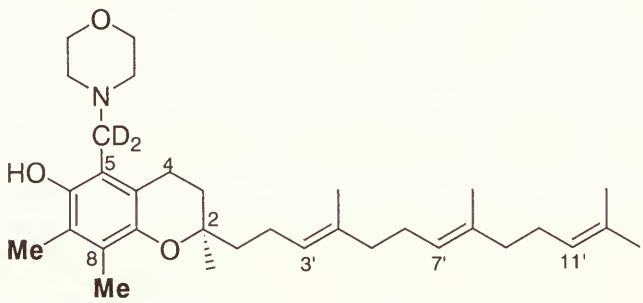


17 **1**



Morpholine (500 μL, 5.43 mmol) and $(CD_2O)_n$ (94.3 mg, 2.94 mmol) were mixed in a round bottom flask and heated at 75°C in oil bath for 1 hour. γ-Tocotrienol (972 mg, 2.37 mmol) was added to the mixture and the temperature was increased to 130°. The reaction was stirred at 130°C for 9 hours. TLC (hexane: EtOAc/ 9:1) showed that no starting material was left. Column chromatography with the same solvent system gave the aminomethyl-d_2 γ-tocotrienol (968.3 mg, 80%).

Aminomethyl-d_2 γ-tocotrienol:



TLC R_f=0.19 (hexane/EtOAc=9:1)

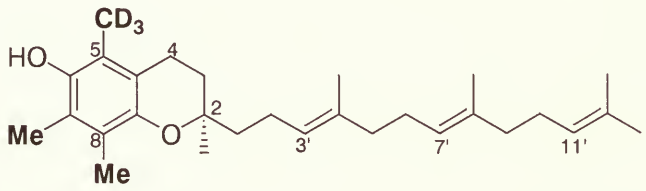
^{1}H-NMR ($CDCl_3$) 5.12(m, 3H, C3'-H, C7'-H, C11'-H), 3.78(br, 4H, morphline-O-CH_2), 2.63(m, 4H, morphline-N-CH_2), 2.63 (m, 2H, C4-CH_2), 2.17(s, 3H, Ar- CH_3), 2.14(s, 3H, Ar- CH_3), 2.09-2.2 (m, 10H, CH_2), 1.81(m, 2H, C3-CH_2), 1.71(s, 3H, CH_3), 1.61(s, 9H, $3CH_3$), 1.50-1.75(m, 2H, CH_2), 1.26(s, 3H, CH_3).

^{13}C-NMR ($CDCl_3$) 148.92, 144.74, 135.49, 135.34, 131.64, 125.64, 124.77, 124.72, 124.56, 123.12, 116.40, 114.74, 77.64, 74.48, 67.17, 53.12, 40.10, 40.08, 31.93, 27.13, 26.97, 26.11, 24.01, 22.58, 20.85, 18.09, 16.40, 16.27, 12.29, 12.15.

MS[EI+] m/z 430(100%), 205(10%), 165(90%).

N-morpholinomethyl-d_2 γ-tocotrienol (968.3 mg, 1.89 mmol) was mixed with $NaCNBD_3$ (563.9 mg, 8.56 mmol) in *i*-BuOH 15 mL. The reaction mixture was heated to reflux for 6.5 hours, and then quenched with 2M HCl until no gas evolution was evident. The mixture was extracted with Et_2O (3 x 10 mL). The ethyl ether layer was washed with saturated $NaHCO_3$ solution, dried over $MgSO_4$ and concentrated *in vacuo*. Column chromatography using hexane: EtOAc/ 9:1 gave 5-trideuteromethyl-α-tocotrienol as yellow oil (613 mg, 76%).

4.3.5 Analytical data of 5-trideuteromethyl-α-tocotrienol:



TLC R_f=0.45 (hexane/EtOAc=9:1)

^{1}H-NMR ($CDCl_3$) 5.14(m, 3H, C3'-H, C7'-H, C11'-H), 4.25(s, 1H, OH),

2.63(t, 2H, *J*=7Hz, C4-CH_2), 2.17(s, 3H, Ar- CH_3), 2.13(s, 3H, Ar-CH_3), 2.13(m, 2H, C2'-CH_2), 2.12(s, 3H, Ar- CH_3), 2.10(m, 4H CH_2), 1.99(m, 4H, CH_2), 1.80(m, 2H, C3-CH_2), 1.68(s, 3H, CH_3), 1.65,1.55(m, 1H of each, C1'-CH_2), 1.62(m, 6H, C12'-2CH_3), 1.60(s, 3H, CH_3), 1.26(s, 3H, C2-CH_3).

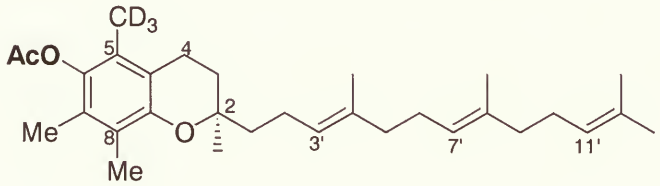
^{13}C-NMR ($CDCl_3$) 145.88, 145.00, 135.44, 135.35, 131.66, 124.81, 124.61, 123.02, 121.45, 118.82, 117.72, 74.68, 40.12, 40.10, 39.91, 32.01, 27.15, 26.99, 26.11, 24.12, 23.08, 21.45, 18.09, 16.40, 16.30, 14.54, 12.61, 12.19,10.93.

MS[EI+](%) m/z 427(M+, 98.8), 191(23.2), 168(80.9), 69(100).

HRMS 427.35204

4.4 Synthesis of 5-trideuteromethyl-α-tocotrienol acetate

5-Trideuteromethyl-α-tocotrienol (3.11 g 7.27 mmol) was mixed with 5 eq of acetic anhydride and 1.05 eq of sodium acetate. The mixture was stirred and heated to reflux for 6 hours. The mixture was cooled to room temperature, water was added. The mixture was neutralized with 5% $NaHCO_3$ until pH=7. The aqueous layer was extracted with $CHCl_3$ (3 x 50 mL), and organic layer was dried over anhydrous $MgSO_4$ followed by concentration under reduced pressure. Column chromatography using 10% EtOAc in hexane gave product 2.49 g (yield 73%).



5-CD_3-α-tocotrienol acetate

TLC R_f=0.52 (hexane/EtOAc=9:1)

^{1}H-NMR ($CDCl_3$) 4.96(m, 3H, C3'-H, C7'-H, C11'-H), 2.44(t, 2H, *J*=7Hz, C4-CH_2), 2.16(s, 3H, OCO-CH_3), 1.94(s, 3H, Ar-CH_3), 1.86(s, 3H, Ar-CH_3), 1.85(m, 10H, CH_2), 1.64(m, 2H, C3-CH_2), 1.53(s, 3H, CH_3), 1.44 (s, 6H, $2CH_3$), 1.43(s, 3H, CH_3), 1.38(m, 2H, C1'-CH_2), 1.10(s, 3H, C2-CH_3).

^{13}C-NMR ($CDCl_3$) 169.98, 149.79, 141.12, 135.45, 135.28, 131.49, 127.12,

124.92, 124.81, 124.69, 123.45, 117.71, 75.20, 40.18, 31.57,

27.24, 27.24, 27.06, 26.16, 24.44, 23.17, 22.68, 21.04, 20.88,

18.12, 16.44, 16.33, 14.62, 13.37, 12.28, 11.73.

MS[EI+](%) m/z 469(41), 427(77), 168(65), 69(100).

4.5 Synthesis and Radioactivity Analysis of 5-^{14}C-methyl-α-tocotrienol:

4.5.1 Preparation:

^{14}C-Paraformaldehyde $(^{14}CD_2O)_n$ (250μCi with radioactivity of 1.9 Ci/g, 0.13mg) was bought from American Radiolabelled Chemical Inc.(St. Louis, MO). Non-radiolabelled paraformaldehyde $(CD_2O)_n$ (2.6 mg, 0.087 mmol) and $(^{14}CD_2O)_n$ (0.13 mg, 0.00406 mmol) were mixed in a 2mL vial, morpholine (30 μL, 0.34 mmol) was added to this vial and heated at 75°C in oil bath for 1.5 hour. γ-Tocotrienol (200 mg, 0.487 mmol) was added to the mixture and the temperature was increased to 130°. The reaction was stirred at 130°C for 4.5 hours. Cooling to room temperature followed by column chromatography with hexane: EtOAc / 4:1 gave the ^{14}C-labelled N-morpholino-γ-tocotrienol.

The above ^{14}C labelled N-morpholinomethyl- γ-tocotrienol was mixed with $NaCNBH_3$ (31.4 mg, 0.5 mmol) in *i*-BuOH 0.6 mL in a 5 mL "V" shaped bottom vial. The vial was sealed tightly. The reaction mixture was heated to reflux for 5.5 hours, and then quenched with 10% HCl until no gas evolution. The mixture was extracted with Et_2O (2.5 mL x 3). The ethyl ether layer was neutralized with saturated $NaHCO_3$ solution, dried over $MgSO_4$ and concentrated under N_2 flow. Preparative TLC chromatography using hexane: EtOAc/ 9:1 gave 5-^{14}C-methyl-α-tocotrienol as light yellow oil (12.4 mg) with an overall yield of 27% from the starting $(^{14}CD_2O)_n$.

4.5.2 Radioactivity Analysis:

The above 5-^{14}C-methyl-α-tocotrienol was dissolved in 25 mL solvent mixture of 1:1 Toluene : EtOH. 2μL of this solution was taken for the radioactivity test by using scintillation counter and given the radioactivity value of 36578.67 DPM.

Thus, the radioactivity value of the total product is calculated as following:

(36578.67 DPM/ 2 μL) x (25 x 10^3 μL/2 μL) / (2.22 x 10^{12} DPM/Ci) = 205.7 μCi

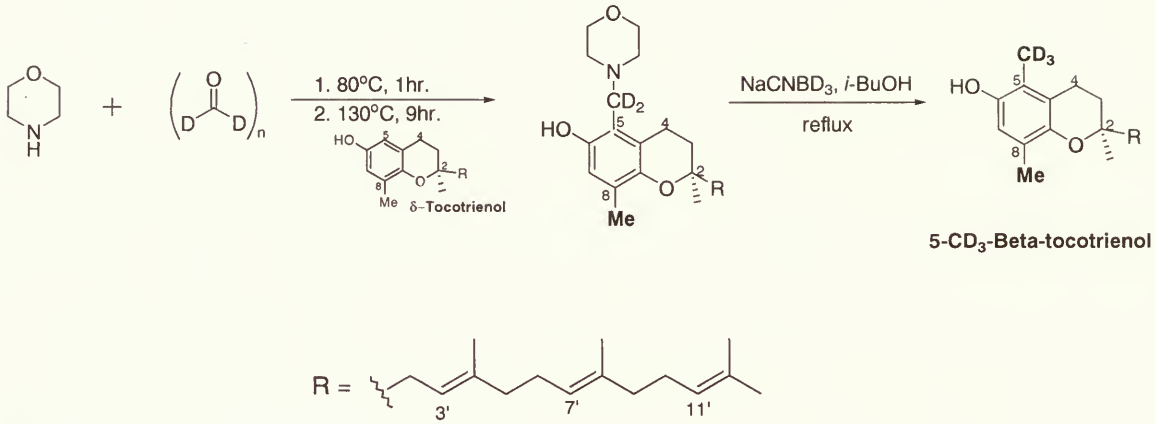
205.7μCi in 12.4 mg of radiolabelled 5-^{14}C-methyl-α-tocotrienol, its specific activity is 0.7mCi/mmole.

The percentage radioactivity of this product over the starting material $(^{14}CD_2O)_n$ is:

205.7 μCi/ 250 μi = 82%

This means that 82% radioactivity of the starting material $(^{14}CD_2O)_n$ was retained in the product 5-^{14}C-methyl-α-tocotrienol. But it is impossible to have an 82% radiochemical yield and a 27% chemical yield at the same time. After we contacted the supplier, American Radiolabelled Chemical Inc., the company made a comment about this. Sometimes, the specific activity of they sent to customers is higher than they wrote on the technical data sheet. In our case, instead of 250μCi, the supplier provided us 205.7 μCi / 27% = 762 μCi of ^{14}C-Paraformaldehyde.

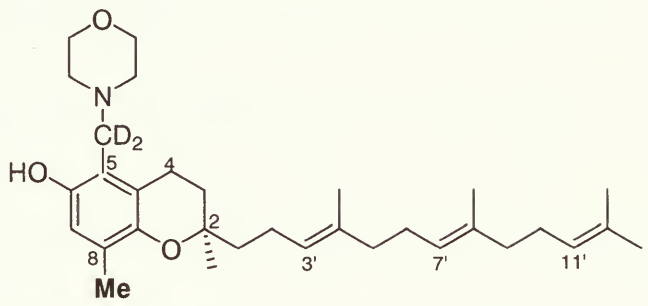
4.6 Synthesis of 5-trideuteromethyl-β-tocotrienol:



4.6.1 Preparation N-morpholinomethyl-d_2-β-tocotrienol

Morpholine (100uL, 1.26mmol) and $(CD_2O)_n$ (4mg, 0.125mml) were mixed in a round bottom flask and heated at 80° in oil bath for 1 hour. δ-Tocotrienol (49.3 mg, 0.12 mmol) was added to the mixture and the temperature was increased to 130°. The reaction was stirred at 130° for 16 hours. TLC using hexane: EtOAc/ 9:1 showed no starting materials left. Silica gel column chromatography with the same solvent system gave the N-morpholinomethyl-d_2 β-tocotrienol (39.9 mg, 65%).

N-morpholinomethyl-d_2 β-tocotrienol



TLC R_f=0.15 (hexane/EtOAc=9:1)

^{1}H-NMR ($CDCl_3$) 6.56(s, 1H, Ar-H), 5.12(m, 3H, C3'-H, C7'-H, C11'-H), 3.78(br, 4H, morphline-O-CH_2), 2.63(m, 6H, 4H from morphline-N-CH_2; 2H from C4-CH_2), 2.14(s, 3H, Ar-CH_3), 2.09-2.2 (m, 10H, CH_2), 1.81(m, 2H, C3-CH_2), 1.71(s, 3H, CH_3), 1.61(s, 9H, 3CH_3), 1.50-1.75(m, 2H, C1'-CH_2), 1.26(s, 3H, C2-CH_3).

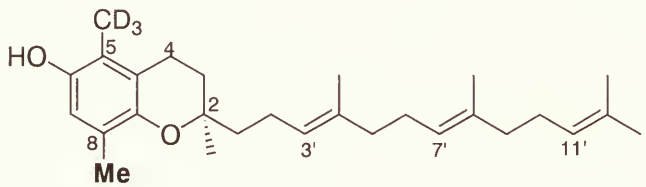
^{13}C-NMR ($CDCl_3$) 150.39, 145.26, 135.48, 135.34, 131.63, 127.31, 121.41, 124.98, 124.77, 124.55, 119.15, 116.96, 115.38, 77.65, 75.68, 74.52, 67.15, 53.22, 53.14, 40.10, 39.81, 31.83, 27.13, 26.96, 26.11, 24.05, 22.85, 20.97, 18.09, 16.51, 16.40, 16.26, 11.91.

MS[EI+](%) m/z 424(73), 410(68), 165(97), 69(100).

4.6.2 Preparation of 5-CD_3-β-tocotrienol

N-morpholinomethyl -d_2 β-tocotrienol (39.9 mg, 0.08 mmol) was mixed with $NaCNBD_3$ (26.3mg, 0.4mmol) in *i*-BuOH 1mL. The reaction mixture was heated to reflux for 6 hours, and then quenched with 2 M HCl until no gas evolution was visible. The mixture was extracted with Et_2O (3 x 10 mL). The ethyl ether layer was washed with saturated $NaHCO_3$ solution, dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Column chromatography using hexane: EtOAc/ 9:1 gave 5-CD_3-β-tocotrienol as yellow oil (17.2mg, 52%).

5-CD_3-β-tocotrienol



TLC R_f=0.32 (hexane/EtOAc=9:1)

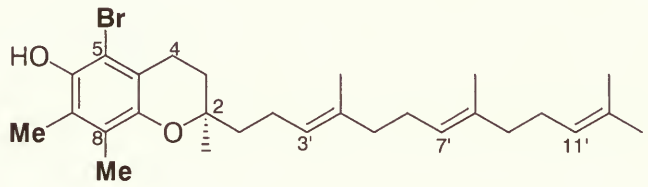
^{1}H-NMR ($CDCl_3$) 6.46(s, 1H, Ar-H), 5.08(m, 3H, C3'-H, C7'-H, C11`-H), 2.59(t, 2H, *J*=7Hz, C4-$CH_2$), 2.10(s, 3H, Ar- $CH_3$), 2.00(m, 10H $CH_2$), 1.80(m, 2H, C3-$CH_2$), 1.68(s, 3H, $CH_3$), 1.65(m, 2H, C1`-CH_2), 1.62(m, 6H, 2CH_3), 1.55(m, 3H, CH_3), 1.30(s, 3H, C2-CH_3).

^{13}C-NMR ($CDCl_3$) 146.29, 146.12, 135.48, 135.35, 131.66, 124.78, 124.73, 124.57, 124.46, 120.69, 119.46, 115.67, 74.64, 40.07, 39.77,

	31.81, 27.13, 26.97, 26.09, 24.15, 22.56, 21.16, 18.08, 16.38, 16.24.
MS[EI+](%)	m/z 413(M+, 70), 194(24), 154(90), 69(100).
HRMS	413.33668

4.7 Preparation of 5-Bromo-α-tocotrienol

γ-Tocotrienol (125.8 mg, 0.31 mmol) and $PyHBr_3$ (121.2 mg, 0.38 mmol) were mixed in dry benzene (3 mL) in a round bottom flask at 0°C. The reaction mixture was stirred at 0°C for 14 hours and then warmed to room temperature. Water was added to the reaction mixture, and the aqueous layer was extracted with hexane (2 x 10 mL). The organic layers were separated and dried over anhydrous $MgSO_4$ and concentrated in *vacuo*. Silica gel column chromatography using 2% EtOAc in hexane gave product 80.0 mg (53%).



12

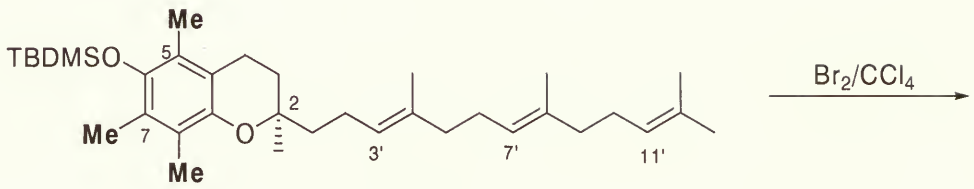
TLC R_f=0.32 (hexane/EtOAc=9:1)

^{1}H-NMR ($CDCl_3$) 5.21(s, 1H, OH), 5.11(m, 3H, C3'-H, C7'-H, C11'-H), 2.69(t, 2H, *J*=7Hz, C4-CH_2), 2.28(s, 3H, Ar-CH_3), 2.12(s, 3H, Ar-CH_3), 2.10 (m, 6H, CH_2), 1.95(m, 4H, CH_2), 1.83(m, 2H, C3-CH_2), 1.71(s, 3H, CH_3), 1.61(s, 9H, $3CH_3$), 1.50-1.75(m, 2H, C1'-CH_2), 1.26(s, 3H, C2-CH_3).

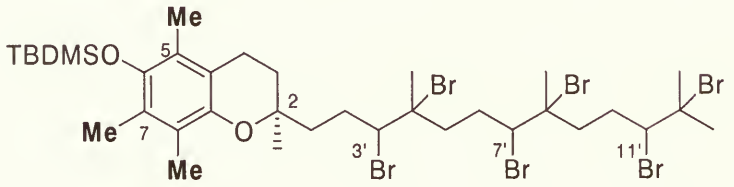
^{13}C-NMR ($CDCl_3$) 149.75, 146.20, 143.78, 135.62, 135.37, 131.64, 125.80, 124.54, 120.27, 122.76, 117.66, 109.66, 77.63, 75.56, 49.37, 40.10, 39.97, 38.04, 31.90, 27.14, 26.96, 24.44, 24.01, 23.88, 18.10, 16.29, 13.32, 12.26

MS[EI+](%) m/z 490(M+2, 31), 488 (M+, 31), 229 (38), 69(100).

4.8 Preparation of compound 37

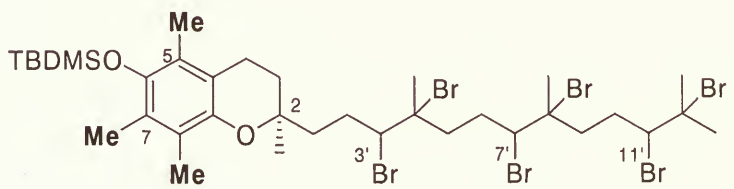


29



37

Br_2 (84μL, 1.64mmol) in CCl_4(1 mL) was slowly added to the solution of TBDMS- α-tocotrienol (290mg, 0.54mmol) in CCl_4 (2 mL) under argon flow over 30min. The reaction mixture was stirred overnight at room temperature and then condensed in *vacuo* to give the product **37** as thick orange oil (351mg, 64%).



37

TLC R_f=0.48 (hexane/EtOAc=9:1)

^{1}H-NMR ($CDCl_3$) 3.9(br, 3H, C3'-H, C7'-H, C11'-H), 2.55(m, 2H, C4-CH_2), 2.0 (s, 3H, Ar-CH_3), 1.95(s, 3H, Ar-CH_3), 1.88(s, 3H, Ar-CH_3), 1.80~1.55(m, 12H, 6CH_2), 1.70, 1.45(m, 2H, C3-CH_2), 1.65(s, 6H, 2CH_3), 1.55(s, 6H, 2CH_3), 1.1 (s, 3H, C2-CH_3), 0.87(s,

9H, *t*Butyl-CH_3), 0.0(s, 6H, 2 dimethyl-CH_3).

MS[EI+](%) m/z 1018(M+, 100%), 1016(73%), 1017(31%), 1019(42%), 1020(79%).

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